STUDY OF ANALYTICAL TECHNIQUES IN PLANETARY QUARANTINE

Final Report

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Prepared by

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ABSTRACT

This report summarizes analyses related to two major areas of the NASA Planetary Quarantine Program, viz,

1. Methods for the formulation of planetary quarantine standards and for the definition of measures of compliance. Emphasis in this area is placed upon the simplification and clarification of several concepts within the quarantine requirements framework, as well as a sensitivity study of program implementation.

2. Analytical techniques related to the heat sterilization of planetary spacecraft. Emphasis in this area is placed upon survival model development, evaluation of experimental data, model parameter estimation, the sterilizing effects of heat-up and cool-down and the feasibility of a physical diffusion model of microbial resistance to heat sterilization.

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I. INTRODUCTION

This is the final report under contract NASw-1550, carried out by Exotech Incorporated for the NASA Headquarters, Office of Biosciences. It summarizes the results of work accomplished during the twelve month period beginning December 7, 1966.

The material presented herein is partitioned into two major parts. The first, Section II, deals with methods for the formulation of planetary quarantine standards and measures of compliance. For the work reported herein, emphasis in this area was placed upon:

1. simplification of the analytical framework to permit effective use at the level of international discussions;
2. a more precise distinction between quarantine parameters which can be made subject to international agreements and those which should not be so constrained; and,
3. establishment of the sensitivity of the planetary program implementation to specific choices of quarantine parameters.

The second part, Section III, of this report deals with the development and evaluation of analytical techniques related to the heat sterilization of planetary spacecraft. Work reported in this area reflects an emphasis on:

1. establishing the dependency of microbial survival model parameters on population and environmental characteristics;
2. estimation of survival model parameters;
3. evaluation of sterilizing effects due to heat-up and cool-down when models other than the logarithmic survival model are used; and,
4. feasibility of a physical diffusion model of microbial resistance to heat sterilization.

A significant portion of the work performed under this contract was either published or disseminated to interested individuals or organizations in the course of the program. In view of this, the approach taken to the preparation of this report is that of assembling existing documents, generated under the contract, in the form of appendices to this report while focusing in the main body on a discussion which relates the various appendices to the objectives of the program. Summary discussions, aimed at assessing progress made toward achieving program objectives, are also included. In this connection, it is to be noted that the subject matter of Sections II and III (and their associated appendices) are relatively independent of one another and can be read separately.

Samuel Schalkowsky was principal investigator on the work reported herein. Specific tasks were carried out in collaboration with the following personnel: Dr. Robert Wiederkehr (of Westat Research Incorporated) provided consultation in statistics and made major contributions in the development of the log-normal model for microbial survival and in the modelling associated with the development of quarantine standards; Saul Honigstein contributed to the analyses and theoretical developments in all aspects of the program; M. Barrett studied the feasibility of a physical diffusion model for microbial survival; and, Robert Kline, who joined Exotech toward the end of this program, contributed to the statistical analyses relating to microbial survival models.

II. PLANETARY QUARANTINE REQUIREMENTS - CRITERIA AND MEASURES OF COMPLIANCE

Work described in this section relates to activities of the Committee on Space Research (COSPAR) of the International Council of Scientific Unions concerning the formulation of standards for planetary quarantine.

Based on recommendations of a Panel on Standards for Space Probe Sterilization of the COSPAR Committee on Potentially Harmful Experiments in Space, resolutions are periodically issued by COSPAR setting forth recommended standards for planetary quarantine. In view of U. S. participation in COSPAR and its desire to abide by COSPAR resolutions, planetary quarantine programs carried out by NASA must be consistent with the above resolutions. Conversely, quarantine standards set by COSPAR should reflect applicable developments in the NASA quarantine program. Work performed by Exotech Incorporated under the subject contract was oriented towards the latter goal by examining approaches to the formulation of quarantine standards, evaluating their implications on NASA programs, and providing NASA with quantitative and qualitative information on preferred international standards for planetary quarantine.

The focal point for COSPAR activities in planetary quarantine is its annual meeting. Most of the work reported herein was concentrated in approximately a six month period preceding, and including, the July 1967 meeting of COSPAR held in London, England. This work was greatly influenced by the following:

(a) The analytical framework (model) used by U. S. representatives during the 1966 COSPAR discussions in Vienna, and included (by reference) in the resulting resolutions, was criticized by the Space Science Board of the National Academy of Sciences as being inadequate and inappropriate for future use. (This model had been developed by Exotech Incorporated under an earlier contract with NASA (1)*.) Since the National Academy of Sciences is the official

* Numbers in parenthesis denote references which are listed separately.

U. S. representative at COSPAR, the above criticisms led to a detailed review by NASA of analytical models for the formulation of planetary quarantine standards.

(b) During its 1966 deliberations in Vienna, the COSPAR Committee on Potentially Harmful Experiments in Space recommended that a standard nomenclature be established for future use in the evaluation of planetary contamination probabilities. Representatives of NASA were called upon to assist in implementing this recommendation through participation in a subcommittee organized for that purpose (2).

Work performed under the subject contract in relation to the above, and including associated technical activities, is summarized in the remainder of this section.

A. Review of Mathematical Modelling

The first organized review of modelling for planetary quarantine standards was undertaken by an ad hoc subcommittee of the American Institute of Biological Sciences (AIBS) Spacecraft Sterilization Advisory Committee. The group met under the chairmanship of Dr. Richard Cornell at the Florida State University in Tallahassee, February 8 and 9, 1967. Samuel Schalkowsky of Exotech was a member of this subcommittee and actively participated in its work. As described in the minutes of these meetings (3), major objectives were:

(1) "to outline a mutually agreeable approach to the Planetary Quarantine Program considering our international agreements; and,

(2) to discuss objectives, assumptions and considerations occuring in specific models used in the Planetary Quarantine Program with a view toward arriving at a mutually agreeable set of each. "

The meetings were very useful from the point of view of exchanging views amongst a diversity of people intimately associated with the NASA Planetary Quarantine Program. However, mutually agreeable conclusions were not determined. Specific contributions by Exotech Incorporated to this review are

contained in the minutes of these meetings (3) and will not be repeated here.

Although no explicit justification was provided, the Space Science Board expressed concern over the adequacy of the existing model for planetary quarantine, considering it to be "naive" and "unrealistic". Recommendations for a critical review specifically suggested that mathematicians versed in probability theory (not previously associated with the program) be called upon for advice. A review meeting was subsequently held by NASA Headquarters on April 25, 1967 and was attended by four mathematicians, two each from the National Institutes of Health and the National Bureau of Standards. The meeting was chaired by Mr. Lawrence B. Hall and attended by Dr. Homer Newell and other key personnel of the Office of Space Science and Applications (OSSA). Exotech Incorporated presented the rationale for the mathematical model used at the 1966 COSPAR meetings and participated in discussions concerning the use of such models in international agreements on planetary quarantine standards. Representatives of Sandia Laboratories also took part and presented views on alternatives to the 1966 model.

Since much of the criticism seemed to evolve from a desire for greater mathematical sophistication, i.e., the feeling that the model is too simple for a complex problem such as planetary quarantine, it appeared desirable to elaborate on the mathematical derivations and to identify the assumptions which led to the relatively simple models used. A summary of a presentation by Exotech to the NASA review meeting, aimed at the above, is provided in Appendix A. However, this material should not be viewed as the sole basis for the recommended approach to the formulation of planetary quarantine standards. Indeed, it is particularly important not to allow sophisticated analysis to obscure problems which are not amenable to such analysis. These considerations were brought out at the NASA review meeting by many of the participants and are noted below. The conclusion of the NASA review was that the present model is basically adequate as a framework for agreements at the level of COSPAR and that greater analytical complexity can not be justified in view of the highly uncertain nature of the parameters which enter into it. It was also recommended that, for the purpose of considering modifications to prior agreements with COSPAR - either with respect to parameters

which are to be made subject to agreements or with respect to the magnitudes of these parameters, sensitivity studies should be conducted using the existing model.

Much of the effort following the above review consisted of developing a suitable basis for the then forthcoming discussions of COSPAR in London and included the recommended sensitivity studies. A number of briefings were made to NASA in which results of Exotech studies were presented. These are most readily described by the charts and accompanying comments contained in Appendix B.

B. Standard Nomenclature

Recommendations submitted by Exotech Incorporated for a standard nomenclature in planetary quarantine were guided by the following considerations:

(1) Technical nomenclature can not be viewed as an entity in itself but must be considered as a tool for clarifying the analysis in which the nomenclature is to be used. The nomenclature recommended by Exotech Incorporated was thus geared to the mathematical models to be used at the level of COSPAR for analysis of planetary quarantine requirements.

(2) One of the primary functions of analysis in the activities of COSPAR member nations, as it relates to planetary quarantine, is the demonstration by a nation of its adherence to agreed upon standards. Documents on standard nomenclature must therefore specifically serve this purpose by providing a common "language" without unnecessarily constraining the particular analytical techniques which can be used.

(3) The scope of the nomenclature to be defined must be broad enough to encompass the consideration of quarantine requirements for planetary programs at various stages of development, e.g., from the formulation of requirements for planets whose exploration is only being contemplated to the demonstration of adherence on accomplished missions.

A document entitled "Recommended Basic Nomenclature for Planetary Quarantine" was prepared in accordance with the above considerations and submitted to NASA towards the end of May 1967. This document underwent some revision at the London COSPAR meeting. The standard nomenclature document which includes these revisions is contained in Appendix C.

C. Summary and Conclusions

As noted earlier, the work described in the preceding sections was oriented to the 1967 COSPAR meeting in London, England. S. Schalkowsky of Exotech Incorporated attended the sterilization symposium associated with the COSPAR meeting to present the paper discussed in Section III of this report. He also had the opportunity to participate in discussions at COSPAR on planetary quarantine standards and related topics. In addition to contributing to working groups of the COSPAR Symposium on Sterilization Techniques, S. Schalkowsky was invited by Professor C. G. Heden, Chairman of the Panel on Standards for Space Probe Sterilization, to participate in meetings of this panel and also to attend a meeting of the COSPAR Consultative Group on Potentially Harmful Effects of Space Experiments which dealt with planetary quarantine.

The basic nomenclature document of Appendix B was accepted by the COSPAR Panel on Standards for Space Probe Sterilization as a recommended tentative guideline for member nations in demonstrating adherence to agreed upon standards. A modified, shortened version of Appendix A was also prepared by Exotech Incorporated to complement the "Basic Nomenclature" document of Appendix B. This document, titled "Analytical Rationale for Basic Quarantine Relationships" was also accepted by the COSPAR Panel on Standards for Space Probe Sterilization for use in conjunction with the model contained in the recommended nomenclature document. Final acceptance of these documents, and specific quarantine standards relating to them, are subject to completion of COSPAR review and approval procedures.

In summary, work performed by Exotech Incorporated under the subject contract has contributed to the introduction of methods for the formulation of

planetary quarantine standards and the definition of measures of compliance which are believed to reflect current needs of the NASA Planetary Quarantine Program. Specifically, the following has been accomplished:

(1) The analytical framework has been sufficiently simplified to permit effective use at the level of COSPAR.
(2) The distinction between quarantine parameters which can be made subject to international agreements and those which should not be so constrained in order to avoid unnecessary interference with program implementation has been brought into sharper focus.
(3) The sensitivity of planetary program implementations to specific choices of quarantine parameters has been established for the range of values considered in the past year.

It is anticipated that future needs in this area will center around the following two considerations:

(1) Emphasis at COSPAR can be expected to shift to the development of detailed procedures for demonstrating adherence by member nations both to the spirit as well as the letter of international agreements. NASA must clearly be a part of these developments in order to assure the compatibility of these procedures with the U. S. Planetary Quarantine Program.
(2) There is increasing recognition that planetary quarantine constraints involve a "cost". There is also a desire to balance this cost against the risks of planetary contamination. The framework of international discussions can therefore be expected to expand to include qualitative and, perhaps, quantitative measures of "costs" vs. benefits relating to alternative choices of quarantine standards.

III. ANALYTICAL TECHNIQUES IN HEAT STERILIZATION

A. Introduction

This section describes work accomplished under Task B of the subject contract and relates to analytical techniques for implementing spacecraft sterilization requirements. Emphasis in the associated analyses was placed upon the development and evaluation of mathematical models which relate attained levels of spacecraft sterility to heat sterilization procedures. In particular, the models considered consist of quantitative expressions for microbial survival probabilities in terms of population and environmental parameters. Special attention was directed towards the determination of model parameters on the basis of empirical data, the integration of lethality over time-varying sterilization temperatures, and the possibility of evolving a physical diffusion model for describing the resistance of microbial spores to sterilizing temperatures.

In the heat sterilization of a spacecraft the particular requirement to be implemented is that the probability of contamination at the conclusion of the heat cycle be less than some prescribed amount. The level of contamination, in turn, is describable in terms of the microbial load on various portions of the spacecraft and corresponding survival curves associated with the resistance of individual microbial spores. Analytical techniques relating to sterilization requirements can therefore be discussed in terms of probabilistic survival curves.

Properly formulated and validated survival curves serve several functions. First, they provide a basis for describing the resistance and survival characteristics of microbial spores under varying population and environmental conditions. They serve as a convenient catalogue of observed microbial resistances, indexed over classes of realistic environmental situations. In addition, survival curves constitute a basic tool for analysis of experimental procedures involving heat sterilization. For example, they can be used in the determination of critical

factors involved in a prescribed sterilization procedure. Finally, survival models can be applied in the prediction of results obtained from various heat sterilization operations. To serve present needs of the NASA Planetary Quarantine Program, consideration must be given to the development, evaluation and application of microbial survival models in all three of these areas, i. e., description, analysis and prediction.

B. Survival Models - Characterization of Experimental Data

The traditionally accepted "law" governing the resistance of microorganisms exposed to a sterilizing environment is that they lose viability exponentially, i. e., the number of survivors decreases by one decade in constant intervals of heating time. The corresponding analytical "exponential" model is expressible by

$$\frac{N(t)}{N_o} = e^{-t/D} \qquad \text{(III-1)}$$

where N_o denotes the initial size of the viable population, N(t) denotes the number of survivors at exposure time t and D denotes a resistance parameter, the "D-value", associated with the particular species and sterilization environment. As indicated in a previous Exotech report (4), experimental data has frequently contradicted this model.

Previously reported efforts of Exotech (4) produced the "two-parameter log-normal" model as an alternate and improved description of laboratory survival data. This characterization specifies that the natural logarithm of the survival time of a microorganism randomly selected from a given population is normally distributed. A mathematical representation of the two-parameter log-normal model is given by

$$\frac{N(t)}{N_o} = 1 - \frac{1}{\sqrt{2\pi}\,\sigma} \int_o^t \frac{1}{x} \exp\left[- \frac{(\ell n\, x - \mu)^2}{2\sigma^2} \right] dx \qquad \text{(III-2)}$$

where μ and σ^2 denote microbial resistance parameters.

The basic assumption underlying the two-parameter log-normal model is that the prior history of heating time affects the future resistance of microorganisms subjected to a sterilizing environment, i. e., that there is a cumulative time effect to

exposure. Work associated with this model produced the conclusion that, in general, it more accurately describes microbial heat resistance than the exponential model. This proved particularly true for relatively long heating times. For this reason, the two-parameter log-normal appears more reliable for extrapolating to survival probabilities associated with heating times greater than those for which experimental data is available. It was also found that, in general, the variance σ^2 is a function only of the type of organism used (and/or the manner in which the samples are prepared), whereas temperature dependence of heat resistance is generally confined to the mean value μ.

In comparing the two-parameter log-normal model with laboratory survival data it was noted that an additional interaction between organisms and their surrounding medium appears to be present. This interaction manifested itself as deviations between the data and the model during an initial period of heating. Further study of this point was made under the present contract and Appendix D of this report contains the principal results of the associated investigation. In particular, it has been determined that the observed biases in the early portions of empirical survival curves can, in principle, be accounted for by the introduction of an additional parameter in the previously discussed two-parameter log-normal model to represent an additional interaction between the spores and the environment. This extension, termed the "three-parameter log-normal" model assumes the form:

$$\frac{N(t)}{N_o} = 1 - \frac{1}{\sqrt{2\pi}\,\sigma} \int_o^t \frac{1}{x+c} \exp\left[- \frac{\left\{ \ell n \left(\frac{x+c}{c} \right) - \mu \right\}^2}{2\sigma^2} \right] dx \qquad \text{(III-3)}$$

where c denotes the added parameter. The end-result of this extension is an additional improvement in the description of survival data. The improvement is principally in the region of lower heating times, i.e., non-zero values of c having little effect on the probabilities associated with long heating times. A study of experimental data indicates that the value of c relates to the physical environment of the spores during sterilization, e.g., whether air-atmospheric, vacuum or nitrogen environments are used, or whether the spores are placed on paper strips, encapsulated in lucite, etc.

C. Empirical Determination of Survival Model Parameters

Associated with each of the previously discussed survival models are various parameters whose values are, in principle, determined by the particular microbial species and sterilizing environment under consideration. Specific parameter values corresponding to a given population and environment can be estimated via conventional numerical fitting procedures on the basis of experimental observations, if appropriate data of this type is available. The previously discussed development and evaluation of survival models was based, in part, upon such determinations. A digital computing routine was developed for the purpose of determining least squares estimates of μ, σ^2 and c for various collections of laboratory survival data. A brief discussion of this computer program is contained in Appendix E of this report. Although improved descriptions of laboratory data were demonstrated via this computer program, more precise quantitative results regarding the parameter values underlying given experimental considerations must await more extensive data than is currently available. Specifically, data should extend over at least six decades of population reduction and have an adequate number of points throughout this range. Further evaluation of computational techniques is warranted on the basis of applications of the existing program and analytical observations discussed in Appendix E.

The sparcity of appropriate laboratory data precluded establishment of a precise functional relationship for the temperature dependence of microbial resistance in the context of the three-parameter log-normal model. On the basis of data shown in Figure 4 of Appendix D it can be hypothesized, however, that the parameters σ^2 and c are relatively insensitive to the temperature of sterilization, i.e., only the mean value, μ, reflects the temperature. In particular, the temperature appears to reflect itself in the parameter μ in the form:

$$\mu = \mu_o + kT^m \qquad \text{(III-4)}$$

where T denotes the sterilizing temperature with μ_o, k and m denoting unknown constants. It is of interest to note that the Arrhenius form of temperature dependence, i.e., where μ would be proportional to $e^{k/t}$, is not consistent with the above

representation even for the limited data now available.

D. Heat-up and Cool-down Effects

Prior studies (1) recognized the need to account for thermal transients during the heat-up and cool-down phases of heat sterilization. Consideration of this point is warranted by the requirements to minimize the destructive effects of heat sterilization on spacecraft components, i.e., the sterilization process should be no more than required to achieve the desired level of sterility. In a previous publication (1), Exotech Incorporated reported on qualitative results of an analysis performed in this regard, assuming an exponential survival curve. In that analysis, the formulation of heat sterilization requirements on the basis of a constant temperature was found inappropriate for achieving a safety factor in sterility assurance. A similar analysis was performed under the present contract for the case where the three-parameter log-normal representation of microbial survival is assumed. Appendix F of this report contains a quantitative formulation of the heat-up and cool-down phases of heat sterilization cycles on survival probabilities. In the development described therein, allowance is made for an arbitrary dependence of the parameter c on temperature, whereas the temperature dependence of μ is assumed to take the form of expression (III-4), above.

For an arbitrarily specified sterilization time, t, the derivation described in Appendix F results in the following expression for the expected porportion of survivors:

$$\frac{N(t)}{N_o} = 1 - \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\alpha(t^* | \mu, \sigma, c)} e^{-x^2/2}\, dx \qquad \text{(III-5)}$$

where

$$\alpha(t^* | \mu, \sigma, c) = \frac{1}{\sigma} \left[\ell n \left(\frac{t^*+c}{c} \right) - \mu \right]$$

$$t^* = c \left[e^{k(T^m - T_1^m)} - 1 + \sum_{i=1}^{n} \frac{1}{c_i} e^{k(T^m - T_i^m)}\, \Delta t_i \right]$$

and where μ, σ and c are the distribution parameters associated with the temperature $T = T(t)$. The partitioned time intervals Δt_i are selected to insure that

$t = t_n$. t^* can be viewed as the exposure time at the constant temperature T which produces the same degree of sterilization as obtained for an exposure for time t at the non-constant temperature $T = T(t)$. The quantities Δt_i denote subintervals of the exposure time interval during which the transient temperature can be assumed constant and equal to T_i $(i = 1, 2, 3, \cdots)$. For the special case where c does not vary with temperature, t^* can be expressed

$$t^* = c \left[e^{k(T^m - T_1^m)} - 1 + \sum_{i=1}^{n} e^{k(T^m - T_i^m)} \Delta t_i \right] \qquad \text{(III-6)}$$

Applications of expression (III-5) or (III-6) to appropriate data should be undertaken and compared with those accomplished for the exponential survival model.

E. Feasibility of a Stochastic Diffusion Model

The two- and three-parameter log-normal models discussed herein are stochastic representations of microbial survival in that they deal with time-varying random processes. These models were based, essentially, on intuitive hypotheses and the justification for their use derives largely from the ability of the resulting model to accurately describe experimental results under a sufficiently large range of test conditions. As an illustration, the log-normal model was shown to be predicated upon the assumptions that survival is dependent upon prior exposure time in a particular functional form. However, there is no explicit connection between this assumption and an hypothesis as to the physical conditions which create the time dependence. Similarly, the stochastic models allow for the existence of a random process with unknown distribution, but no attempt is made to associate this randomness with a particular physical characteristic, e.g., of the spores or the environment.

Work done to date has provided some insight as to the type of functional relationships which are justifiable from an analytical point of view and, at the same time, are also supported by laboratory data. Fortunately, considerable progress was made by others, e.g., Angelotti (5) and Pflug (6), through laboratory investigations to provide a better understanding of the physical processes associated

with the heat inactivation of spores. In particular, attention has been directed to the importance of water activity in heat sterilization, supporting a diffusion process of moisture through the spore walls and surrounding medium induced by the sterilizing temperatures. It thus became pertinent to examine the manner in which progress in the areas of stochastic modelling and physical understanding of the problem can be combined to provide a springboard for a more complete representation of the heat inactivation process under physically meaningful conditions. Such a representation can be termed a "stochastic diffusion model".

On conjectural grounds, a useful connection can be established between the diffusion concept and the log-normal survival model. In particular, if the wall thickness of a large population of spores can be characterized by a normal distribution, then it is possible to show that the time required for the penetration of the wall of a randomly selected spore by a particle of moisture is log-normally distributed. This simple analytical development is more fully discussed in Appendix G of this report. These considerations, along with the desirability of a more complete physical representation of the inactivation process of spores, provides sufficient justification for further development of a "stochastic diffusion" model.

F. Summary

Work described in the present section provided several milestones in the development of an analytical framework essential to the implementation of spacecraft sterilization requirements.

From a descriptive standpoint, the three-parameter log-normal survival model has been demonstrated to accurately characterize microbial survival data under the various environmental conditions which can be encountered in spacecraft sterilization. Insofar as the explicit determination of model parameter values is concerned, more extensive experimental data is necessary. Moreover, such data gathering procedures (laboratory experiments) should be compatible with the objectives of model refinement and parameter determination and evaluation.

The characterization of heat-up and cool-down effects presented herein provides one example of useful analyses available through the use of survival data and

models thereof. Further analysis of this aspect of heat sterilization is warranted.

The log-normal survival model can be expected to be a useful tool in the design and evaluation of heat sterilization procedures, e.g., in sensitivity analyses of survival probabilities in terms of operational parameters. Furthermore, as refinements to the model and associated parameter determination evolve, increased confidence in the prediction of sterility levels will result.

The development of a stochastic diffusion model has been found to be feasible and highly desirable. Such a model, when validated against suitable experimental data, would greatly upgrade the analytical techniques for spacecraft sterilization in the areas of "description", "analysis" and "prediction".

IV. REFERENCES

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5. Angelotti, R. "Protective Mechanisms Affecting Dry Heat Sterilization." Presented at the COSPAR Symposium on Sterilization Techniques, London, England, July 1967.

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APPENDIX A

Presentation by

Dr. R. Wiederkehr

on

AN OPERATIONAL MODEL FOR PLANETARY QUARANTINE REQUIREMENTS

During the period of unmanned exploration of Mars (1965-1985), there will be a number N of launches which will subject Mars to the threat of contamination. These launches may differ in nationality (U.S.A., USSR), type of vehicles, (flybys, orbiters, landers) and many other factors. Because of these differences each launch can be expected to have a different probability of contaminating Mars. Therefore, let the launches be numbered sequentially in time from 1 to N and let p_i be the probability that Mars will be contaminated by the i^{th} launch; and let P_c be the probability that Mars will be contaminated by at least one of these launches during the period of unmanned exploration of Mars. Assuming that contamination of Mars by one launch in no way affects the chances of contamination by another launch, i.e. assuming that the contamination events are independent, one obtains the relationship

$$1 - P_c = \prod_{i=1}^{N} (1 - p_i) \qquad (1)$$

This equation follows immediately from the fact that not contaminating Mars requires that every launch not contaminate Mars.

The goal of the sterilization program is to make the values of the p_i's sufficiently small so that P_c remains less than some prescribed small value (e.g. 0.001), or equivalently that $1 - P_c$ exceeds some value near unity (0.999). From (1) it follows that $p_i < P_c$ so that P_c and all the p_i are very small compared to unity. Under these conditions (1) can be reduced to a very simple expression as will now be shown.

Taking logarithms of (1) yields:

$$\log_e (1 - P_c) = \sum_{i=1}^{N} \log_e (1 - p_i) \tag{2}$$

A Taylor's formula with remainder for $\log (1 - p_i)$ expanded about the point $p_i = 0$ is:

$$\log (1 - p_i) = -p_i + R_{1i} \tag{3}$$

where

$$R_{1i} = -\frac{\varphi_i^2}{2}, \qquad 0 < \varphi_i < p_i \tag{4}$$

Similarly a Taylor's formula for $\log (1 - P_c)$ is:

$$\log (1 - P_c) = -P_c + R_{1c} \tag{5}$$

where

$$R_{1c} = -\frac{\varphi_c^2}{2}, \qquad 0 \leq \varphi_c < P_c \tag{6}$$

Substitution of (3) through (6) into (2) yields:

$$P_c = \sum_{i=1}^{N} p_i + \epsilon \tag{7}$$

where

$$\epsilon = \frac{1}{2} \sum_{i=1}^{N} \varphi_i^2 - \frac{1}{2} \varphi_c^2 \tag{8}$$

The inequalities mentioned above may be summarized as follows:

$$0 < \varphi_i < p_i \leq P_c \tag{9}$$

$$0 < \varphi_c < P_c \tag{10}$$

Substitutions of (9), (10) into (8) gives :

$$-\frac{P_c^2}{2} < \epsilon < \frac{N P_c^2}{2} \tag{11}$$

For example if N is 20 and $P_c = 10^{-3}$, it follows from (11) that $\epsilon < 10^{-5}$. Consequently, the error introduced by ignoring ϵ in (7) is small, and the following equation is recommended as an approximation to (7) and (8):

$$P_c = \sum_{i=1}^{N} p_i \tag{12}$$

The relative error in specifying p_i can be evaluated by using an average p_i, denoted as $\overline{p_i}$. Then, if $\Delta \overline{p_i}$ is the error

$$\frac{\Delta \overline{p_i}}{\overline{p_i}} = \frac{\frac{P_c}{N} - \frac{P_c - \epsilon}{N}}{\frac{P_c - \epsilon}{N}} = \frac{\epsilon}{P_c - \epsilon} \cong \frac{\epsilon}{P_c} = \frac{N P_c}{2} \tag{12a}$$

Thus, in the previous example, for $P_c = 10^{-3}$ and letting N = 20, there would be at most a 1% error in specifying p_i due to the approximation. If the number of launches were to be increased to N = 100, the error would be less than 5%, e.g. the specified $\overline{p_i}$ would be 1×10^{-5} while the "exact" value would fall between 1×10^{-5} and 1.05×10^{-5}. This is clearly an insignificant difference in relation to the basis for choosing $P_c = 10^{-3}$.

Categorization or Aggregation of Launches

In the above discussion each launch was considered separately from all others. It is often convenient to separate the launches into categories such as by nationality, by type of vehicle, etc. and consider the aggregate probabilities of contamination for each category. Toward this end let the N launches be partitioned into k categories, let p_{ij} designate the probability of contaminating Mars by the i^{th} launch of the j^{th} category and let n_j be the number of launches in the j^{th} category. Then (12) can be rewritten as:

$$P_c = \sum_{j=1}^{k} \sum_{i=1}^{n_j} p_{ij} \tag{13}$$

or

$$P_c = n_1 \overline{p_1} + n_2 \overline{p_2} + \cdots + n_k \overline{p_k} \tag{14}$$

where

$$\overline{p_j} \equiv \frac{1}{n_j} \sum_{j=1}^{n_j} p_{ij} \tag{15}$$

In (15) $\overline{p_j}$ is the average probability of contaminating Mars per launch in category j. For example, suppose we are interested in considering only the launches of the U.S. and Russia, and only landers and non-landers. Then let the categories be defined by the following table:

j	Categories	Avg. Probability of contaminating	Number of launches
1	U.S. landers	$\overline{p_1}$	n_1
2	U.S. non-landers	$\overline{p_2}$	n_2
3	Russian landers	$\overline{p_3}$	n_3
4	Russian non-landers	$\overline{p_4}$	n_4

Application of (14) yields :

$$P_c = n_1 \overline{p_1} + n_2 \overline{p_2} + n_3 \overline{p_3} + n_4 \overline{p_4} \qquad (16)$$

Generalization to Include Uncertainty in Number of Launches

In the above formulation the total number of launches N as well as the number of launches in each category n_j were asumed to be known. In reality they are not known but must be predicted and may be considered to be random variables. This amounts to saying that P_c as given by (14) is actually a conditional probability, the condition being that the number of launches in the j^{th} category is n_j for $j = 1, 2, \ldots, k$. To remove this condition, it is necessary to average over all possible values of the n_j's.

If the $\overline{p_i}$'s are assumed to be approximately constant and independent of the n_i's, and (14) is then averaged over all possible values of the n_j's, one obtains :

$$\overline{P_c} = \overline{n_1}\ \overline{p_1} + \overline{n_2}\ \overline{p_2} + \cdots + \overline{n_k}\ \overline{p_k} \qquad (17)$$

where

$\overline{P_c}$ is the average (expected) value of P_c

$\overline{p_i}$ is the average (expected) value of p_i, $i=1, 2, \ldots, k$.

Specialization to the 1966 COSPAR Model

Three additional steps are required to obtain the 1966 COSPAR Model from (17). First, only two categories are considered: landers and non-landers (unsterilized vehicles). This yields

$$P_c = n_L p_L + n_U p_U \tag{18}$$

where n_L = the expected number of landers

p_L = the average probability that a lander contaminates Mars

n_U = the expected number of unsterilized (non-lander) vehicles

p_U = the average probability that an unsterilized vehicle contaminates Mars.

Second, the event that a lander contaminates Mars requires that:

1) at least one viable organism survives the sterilization treatment and arrives at Mars on the lander
2) the viable organisms which survive the sterilization treatment and arrive at Mars are released
3) the viable organisms which survive the sterilization treatment, arrive at Mars and are released also growth and spread.

Let p_N, p_R, and p_G be the probabilities of events 1), 2), and 3). Then

$$p_L = p_N \cdot p_R \cdot p_G \ . \tag{19}$$

Third, the event that an unsterilized vehicle contaminates Mars requires that none of the various possible sources of contamination from an unsterilized vehicle (such as accidental impact, ejecta from attitude control, etc.) actually contaminates Mars. By an argument similar to that leading to (12), it follows that for independent sources of contamination the probability of contamination is the sum of probabilities of contamination due to each such source. For a particular source to contaminate Mars requires that:

1)' the viable organisms due to source i are transferred to the surface of Mars,

2)' the viable organisms transferred to Mars from source i are released

3)' the viable organisms transferred to Mars from source i and released also grow and spread.

Let $(p'_T)_i$, $(p'_R)_i$, $(p'_G)_i$ be the probabilities of events 1)', 2)', 3)'. Then

$$p_U = \sum_i (p'_T)_i (p'_R)_i (p'_G)_i \qquad (20)$$

Substitution of (19) and (20) into (18) yields the 1966 COSPAR Model, namely:

$$P_c = n_L p_N p_R p_G + \sum_i (p'_T)_i (p'_R)_i (p'_G)_i \ . \qquad (21)$$

APPENDIX B

U.S. AGREEMENTS WITH COSPAR

ON

PLANETARY QUARANTINE

Review of considerations pertinent to U.S. position at 1967 COSPAR meetings.

QR
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OBJECTIVES FOR COSPAR AGREEMENTS

1. PROVIDE A DEFINITIVE STATEMENT OF DEGREE OF COMMITMENT TO PREVENTION OF PLANETARY CONTAMINATION.

2. AVOID UNNECESSARY INTERFERENCE WITH IMPLEMENTATION BY LAUNCHING NATIONS.

3. PROVIDE MEANS FOR DEMONSTRATING ADHERENCE TO COMMITMENTS.

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QR- 1

Item 1 : This was predominant consideration for first set of requirements, as developed by Sagan and Coleman for 1964 COSPAR.

Item 2 : Generally, this would be a constraint on first objective, not requiring separate mention. However, over-emphasis in the past on the first objective and rapidly developing implementation technology justify specific emphasis on avoidance of unnecessary interference.

Item 3 : There is particular concern in this country with the sincerity of U.S.S.R. agreements to planetary quarantine commitments. There is therefore also a need to provide means by which U.S.S.R. adherence to commitments can be tested and encouraged.

KEY STEPS IN NASA/SSB - COSPAR AGREEMENTS

1. AGREEMENT ON MODEL AS FRAMEWORK OF DISCUSSION.

2. SELECTION OF MODEL PARAMETERS WHICH WILL BE SUBJECT TO QUANTITATIVE AGREEMENT.

3. AGREEMENT ON NUMERICAL MAGNITUDES OF CHOSEN PARAMETERS.

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QR - 2

Defines scope of presentation.

MODEL CRITERIA

1. SERVE AS A MEANS OF COMMUNICATION IN ACHIEVING OBJECTIVES OF COSPAR AGREEMENTS :
   (a) Define scope of agreement
   (b) Identify constraints which unneccesarily interfere with implementation
   (c) Provide framework for demonstrating adherence

2. COMPLEXITY OF MODEL CONSISTENT WITH STATE OF KNOWLEDGE OF INPUT PARAMETERS.

3. DEVELOPMENT OF MODEL BASED ON ACCEPTED MATHEMATICAL METHODS.

Model is not an end in itself, nor is it a means for demonstrating mathematical sophistication which does not serve the objectives of COSPAR agreements.

1966 COSPAR MODEL

(1) $P_c = n_L \cdot P + n_U \cdot P'$

- P_c: probability that planet will be contaminated
- n_L: number of landers
- P: probability of contamination due to a lander
- n_U: number of non-lander vehicles
- P': probability of contamination due to a non-lander

(2) $P = P_N \cdot P_R \cdot P_G$

- P_N: probability of one viable organism
- P_R: probability of release
- P_G: probability of growth and spreading

(3) $P' = \sum^{i} (P'_T)_i \, (P'_R)_i \, (P'_G)_i$

- $(P'_T)_i$: probability of transferring viable organisms to planet
- $(P'_R)_i$: probability of release
- $(P'_G)_i$: probability of growth and spreading

i = distinct, independent sources of contamination

(4) $P_c = n_L \cdot P_N \cdot P_R \cdot P_G + n_U \cdot \sum^{i} (P'_T)_i \cdot (P'_R)_i \cdot (P'_G)_i$

Describes the model used by U.S. in 1966 COSPAR discussions. Since further analytical justification will follow, only a qualitative rationale is appropriate at this point :

Equation (1) : Assumes P_c to be due to two independent sources:
(a) landers involving sterilized organisms, and
(b) unsterilized vehicles -- orbiter, fly-bys, and launch vehicles for the landers.

An average probability of contaminating the planet, P, is associated with each vehicle in category (a) in conjunction with n_L such vehicles in the estimated period of unmanned exploration. Similarly for P' and n_U.

Since planetary contamination can occur <u>either</u> because of $n_L P$ or $n_U P'$, or both, P_c is given by their sum. The additional term ($- n_L P \cdot n_U P'$) needed for consistency with theoretical formulations of P_c is neglected because it is small compared to either $n_L P$ or $n_U P'$ (neglecting it also makes the assignment of P and P' slightly more conservative).

Equation (2) : Identifies the major events -- presence of a viable organism (P_N), release (P_R) and growth and spreading (P_G), <u>all</u> of which must occur to cause the event associated with P. (The component probabilities P_R and P_G should, strictly speaking, be written as conditional

probabilities but this is not critical to discussions at COSPAR.)

Equation (3) : Analogous to rationale of equation (2) except that

(a) allowance is made for i independent events which may be the source of contamination for one unsterilized vehicle (accidental impact, gaseous ejecta, micro-meteorite spalling, etc.), and

(b) a general term P'_T is provided to define the probability of viable organisms reaching the planet for any one of the independent sources.

Equation (4) : Combines equations (1), (2) and (3).

A DERIVATION OF 1966 COSPAR MODEL

- N LAUNCHES DURING PERIOD OF UNMANNED EXPLORATION

- NOT CONTAMINATING REQUIRES THAT ALL N LAUNCHES NOT CONTAMINATE

- CONTAMINATION BY ONE LAUNCH INDEPENDENT OF OTHERS

$$1 - P_c = (1 - P_1) \cdot (1 - P_2) \cdot \cdots\cdots (1 - P_N)$$

$1 - P_c$	$(1 - P_1)$	$(1 - P_2)$	$(1 - P_N)$
probability of not contaminating Mars	probability that first launch does not contaminate Mars	probability that second launch does not contaminate Mars	probability that N-th launch does not contaminate Mars

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The Equation provides a general statement of the problem for N independent events, each having arbitrary probabilities P_1, P_2 $\cdots$ P_N.

Since this form does not illuminate operational considerations, further manipulations are worth exploring.

AN APPROXIMATION

EXACT EXPRESSION

$$P_c = 1 - \prod_{i=1}^{N} (1 - P_i) = P_1 + P_2 + P_3 + \ldots + P_N + \epsilon$$

APPROXIMATE EXPRESSION (NEGLECTING ϵ)

$$P_c \cong P_c^* = P_1 + P_2 + P_3 + \cdots + P_N$$

ERROR

$$-\frac{P_c^2}{2} < \epsilon < \frac{NP_c^2}{2}$$

The limits of ϵ give the total range of the error due to using the approximate equation

$$P_c = P_1 + P_2 + \cdots + P_N$$

Rather than the basic equation

$$(1 - P_c) = (1 - P_1)(1 - P_2) \cdots (1 - P_N)$$

AN APPROXIMATION

Taking logarithms on both sides of basic equation :

(1) $\ln (1 - P_c) = \ln (1 - P_1) + \ln (1 - P_2) + \cdots\cdots \ln (1 - P_N)$

In general, a Taylor series expansion about $P_i = 0$, truncated after the first term, gives

(2) $\ln (1 - P_i) = -P_i + R_i$ where $R_i = -\frac{\varphi_i^2}{2}$ and $0 < \varphi_i < P_i$

Applying (2) to all the terms of (1) :

(3) $P_c = (P_1 + P_2 + \cdots\cdots P_N) + (R_c - R_1 - R_2 - \cdots\cdots - R_N)$

Let the error be defined as

$$\epsilon = R_c - R_1 - R_2 - \cdots\cdots - R_N = \frac{1}{2}(\varphi_1^2 + \varphi_2^2 + \cdots \varphi_N^2 - \varphi_c^2)$$

Since $P_1, P_2 \cdots P_N \leq P_c$ and $0 < \varphi_i < P_i$

$$-\frac{P_c^2}{2} < \epsilon < \frac{NP_c^2}{2}$$

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EXAMPLES OF MAGNITUDE OF ERROR

$$P_c^* = 10^{-3} \; ; \quad -\frac{P_c^2}{2} < \epsilon < \frac{NP_c^2}{2} \; ; \quad P_c^* - \frac{P_c^2}{2} < P_c < P_c^* + \frac{NP_c^2}{2} \; ;$$

1) For $N = 20$

$$.9995 \times 10^{-3} < P_c < 1.01 \times 10^{-3}$$

2) For $N = 100$

$$.9995 \times 10^{-3} < P_c < 1.05 \times 10^{-3}$$

3) For $N = 200$

$$.9995 \times 10^{-3} < P_c < 1.10 \times 10^{-3}$$

ERRORS ARE NEGLIGIBLE

P_c^* denotes the desired value of P_c which would have been obtained if P_1 , $P_2 \cdots P_N$ were specified by using the basic equation, that is, without the approximation.

Examples show the "actual" values of P_c resulting from the use of the approximation, i.e. the probability of contaminating the planet would be, say, 1.05×10^{-3} rather than the "desired" 1.00×10^{-3}.

SPECIALIZATION TO 1966 COSPAR MODEL

$$P_c = P_1 + P_2 + P_3 + \cdots + P_N$$

CATEGORIZE LAUNCHES AS LANDERS OR NON-LANDERS

$$P_c = \sum_{\substack{i \\ \text{landers}}} P \quad + \sum_{\substack{i' \\ \text{non-landers}}} P'$$

OR

$$P_c = n_L \cdot P + n_U \cdot P'$$

- P' — AVERAGE PROBABILITY OF CONTAMINATION DUE TO A NON-LANDER
- n_U — NUMBER OF NON-LANDER LAUNCHES
- P — AVERAGE PROBABILITY OF CONTAMINATION DUE TO A LANDER
- n_L — NUMBER OF LANDERS

P and P' viewed as the average probabilities in their respective categories of n_L and n_U.

Could also use other aggregations of terms in equation of P_c. For example, it may be convenient to distinguish between the P_i's of U.S. vehicles vs U.S.S.R. vehicles and further categorize each of these into landers and unsterilized vehicles.

One possibility, to be discussed later, is to specify that all P_i have the same value, i.e. that all N missions have an equal probability of contaminating the planet. Denoting this probability as P_m, we would obtain the simple relationship

$$P_c = N P_m \quad .$$

Additional step not shown, but discussed in conjunction with 1966 COSPAR Model, is break-down of P and P' into a product of component probabilities for presence of viable organisms, release, and growth and spreading.

ALTERNATIVE PARAMETER COMBINATIONS FOR INCLUSION IN COSPAR AGREEMENTS

SPECIFY →	P_c or P_c (nation)**	$n_L + n_U$ (total or per nation)	n_L and n_U (total or per nation)	P_G and P'_G
ALTERNATE A	X			
ALTERNATE B	X	X		
ALTERNATE C	X		X	
ALTERNATE D	X			X
ALTERNATE E	X	X		X
ALTERNATE F*	X		X	X

* BASIS OF 1966 COSPAR DISCUSSIONS, USING P_c

** BASED ON $P_c = P_c(\text{U.S.A.}) + P_c(\text{U.S.S.R.}) + P_c(\text{OTHERS})$

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Recommend that 1966 COSPAR Model be retained as basic framework for discussion. However, its presentation to 1967 COSPAR should include better analytical justification and a clearer definition of terms to avoid criticism of the kind voiced in the past year.

In terms of this model, chart shows the matrix of possibilities for the parameters which could be made subject to COSPAR agreements. Note that P_R and P'_R were not subject to COSPAR agreements in 1966 and it is not proposed to change this in 1967.

Before attempting to select one of the shown alternatives, it will be useful to review 1966 agreements and to re-examine the relative significance of the various parameters. This is done in the charts which follow.

1966 COSPAR AGREEMENTS

U.S.A. RECOMMENDATION:

$$P_c < 10^{-3} \; ; \quad n_L = 70 \; ; \quad n_U = 30 \; ; \quad P_G = 10^{-3} \; ; \quad P'_G = 1$$

COSPAR RESOLUTION : (Information Bulletin No. 33, October 1966) :

"It is suggested, therefore, that the basic probability of 1×10^{-3} that a planet will be contaminated during the period of biological exploration continues to be accepted as the guiding criterion for the exploration of Mars, or other planets deemed important for the investigation of extra-terrestrial life of precursors or remnants thereof."

"The computations ought to assign a probability of not less than 10^{-3} that organisms that have gone through a sterilization procedure before landing on the surface of a planet will grow and spread. This change from an inferred 10^{-2} probability in the COSPAR model of 1964 should, however, not be incorporated into the overall probability until the general formation has been agreed upon. The similar value for organisms that have not been exposed to a sterilization procedure should be taken as a unity."

"Members of COSPAR should make available to it, within three months after launch, the sterilization procedures and computations used for each flight to assure prevention of contamination of the planet consistent with the probability of 10^{-3} for the period of biological exploration."

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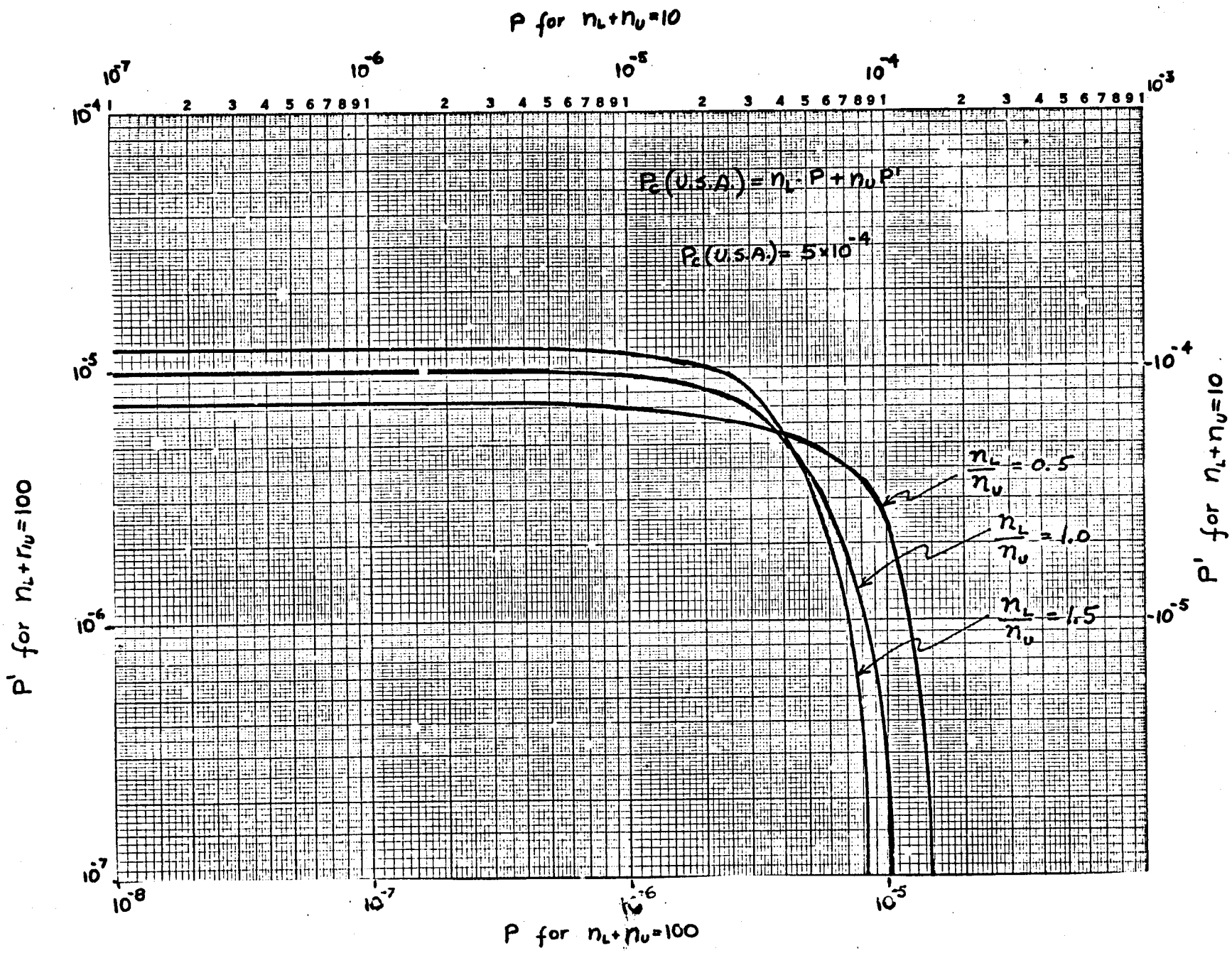
"Formation" should probably be "formulation".

COSPAR statement suggests that $P_G = 10^{-3}$ is not really an accepted number as yet.

U.S. recommendations for n_L and n_U do not enter into COSPAR resolutions.

Last paragraph shows need for an agreed upon model as a basis for submitting computations which are consistent with original formulation of requirements.



ALLOCATION OF PROBABILITY OF CONTAMINATION BETWEEN LANDERS AND NON-LANDERS

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As a first step, we wish to establish sensitivity of P and P' to P_c, n_L and n_U. In all cases, use P_c (U.S.A.) = 5×10^{-4}.

(1) Assume $n_L + n_U = 100$: Use left and bottom scales. Let $n_L / n_U = 1.0$, i.e. $n_L = 50$, $n_U = 50$. Graph shows that if it was desired to favor, say, P' at the expense of P, it would not pay to make $P \ll 10^{-6}$ because P' stays flat at about $P' \approx 10^{-5}$ for smaller values of P.
Similar argument applies if P is to be favored at the expense of P': would make $P \approx 7 \times 10^{-6}$ and $P' \approx 10^{-6}$.
Conclusion: allocation between P and P' for fixed values of P_c, n_L and n_U permits a shifting of up to one order of magnitude in their relative values.

(2) Effect of assuming different ratios of n_L / n_U is not very significant as it changes the limiting values of P or P' by much less than one order of magnitude. Furthermore, if a balanced allocation is to be made between P and P', i.e. if one were to operate in the knee of the curves, the graphs merge there in a narrow region, indicating insensitivity to the choice of n_L/n_U.

(3) If $n_L + n_U$ is 10 rather than 100, would use the top and right side scales. Comparison with opposite scales,

used before for $n_L + n_U = 100$, shows this to simply require a proportional change in P and P'.

Conclusion: $n_L + n_U$ are likely to be in the range of 10 to 100 and the effect on P and P' is therefore restricted to one order of magnitude.

SENSITIVITY OF P OR P'

ASSUME EITHER P OR P' IS FAVORED IN ALLOCATION.

(a) CHANGE IN P OR P' IF P_c IS CHANGED FROM

$P_c = 10^{-3}$ to P_c (U.S.A.) = 5×10^{-4} ..... -0.5 orders of magnitude

(b) CHANGE IN P OR P' IF $n_L + n_U$ IS CHANGED FROM

100 (1966 COSPAR) to 50 (U.S.A.) ..... +0.5 orders of magnitude

EFFECTIVE RANGE OF ALLOCATION FREEDOM.... 1 order of magnitude

A summary of sensitivity of P and P' to parameters likely to be considered at COSPAR.

To proceed with examination of sensitivity in the implementation of P and P', it it reasonable to associate a one order of magnitude effect on P and P' due to agreements on P_c, n_L and n_U.

IMPLEMENTATION OF CONSTRAINTS ON LANDERS

$$P = P_N \cdot P_R \cdot P_G$$

FOR HEAT STERILIZATION : $P_N \cong N_o \cdot 10^{-t/D}$

N_o - INITIAL MICROBIAL BURDEN : BIO-ASSAY, ANALYTICAL ESTIMATION

D - MICROBIAL RESISTANCE VALUE : LABORATORY TESTING AS A FUNCTION OF MEDIUM AND ENVIRONMENTAL PARAMETERS

t - HEAT STERILIZATION TIME : EFFECT ON EQUIPMENT PERFORMANCE AND RELIABILITY

P_R AND P_G READILY DEFINABLE IN TERMS OF ONE VIABLE ORGANISM

This formulation of P presupposes terminal heat sterilization of entire lander vehicle (current approach).

Formulation of P_N is based on exponential kill rate. This is not consistent with experimental data and better models are under study. However, the form shown is adequate for present purposes.

Definitions of terms includes brief statements of related R & D programs.

Sensitivity to COSPAR agreements :

(1) Previously established that P can vary by one order of magnitude as a function of agreements.

(2) Initial burden, N_0, cannot be accurately established and the uncertainty in the value ultimately used will be considerably more than one order of magnitude, e.g. $10^{-3} < N_0 < 10^{8}$. Conclusion: The effect of COSPAR agreements is essentially lost in the choice of N_0 which is not subject to agreements.

(3) There is also considerable uncertainty in the selection of a suitable D value which, because it appears in the exponent, also tends to overshadow one order of magnitude variation in P.

(4) One order of magnitude change in P is equivalent to about 15% change in the sterilization time t. It is unlikely that spacecraft equipment will be so sensitive that 15% change in sterilization time

will make the difference between high reliability or failure.

(5) Effect of P_G is significant if the issue is whether it is 1 or 10^{-3}. However, one order of magnitude in P_G is not overly significant, for the same reasons discussed for P.

P_G and P_R could be defined clearly and simply in terms of one viable organism because in a sterilized spacecraft there will either be one viable organism or none. Probabilities of more than one are relatively insignificant.

ALTERNATE FORMULATION FOR LANDER STERILIZATION

(1) $$P = (P_N)_e \cdot (P_R)_e \cdot (P_G)_e + (P_N)_o \cdot (P_R)_o \cdot (P_G)_o$$

e subscript - denotes organisms embedded inside components and materials

o subscript - denotes organisms on outside surfaces of equipment

If $(P_R)_e \ll (P_R)_o$

then could make sterilization requirement $(P_N)_e > (P_N)_o$

This formulation reflects the approach which distinguishes between internal and external contamination.

Horowitz's suggestion that components not be internally sterilized requires the following :

(a) $(P_N)_e \cdot (P_R)_e \cdot (P'_G)_e < 10^{-6}$ say 10^{-7}

(b) No sterilization implies $(P_N)_e = 1$

(c) Hence $(P_R)_e \cdot (P'_G)_e \leq 10^{-7}$

(d) If $(P'_G)_e$ is taken as 1, in line with current COSPAR recommendations for organisms not subject to sterilization, then this approach requires

$$(P_R)_e \leq 10^{-7}$$

Note : P_R has not been subject to agreement at COSPAR. Hence, justification for $(P_R)_e < 10^{-7}$ is up to us.

This approach would be enhanced by values of P'_G less than unity.

IMPLEMENTATION OF CONSTRAINTS ON UN-STERILIZED VEHICLES

$$P' = \sum^{i} (P'_T)_i \cdot (P'_R)_i \cdot (P'_G)_i$$

MAJOR SOURCES OF CONTAMINATION (P'_T) :

1. ACCIDENTAL IMPACT
2. LANDER RECONTAMINATION
3. EJECTA FROM ATTITUDE AND VELOCITY CONTROL JETS
4. EJECTA FROM MICRO-METEORITE IMPACTS

ASSESSMENT OF $(P'_T)_i$ ALSO LEADS TO ESTIMATION OF AN ASSOCIATED NUMBER OF VIABLE MICRO-ORGANISMS.

P'_R A FUNCTION OF NUMBER OF VIABLE ORGANISMS.

P'_G A FUNCTION OF NUMBER OF VIABLE ORGANISMS ?

Principal effects of constraints are :

(1) Need to bias trajectory and hence provide a capability for multiple mid - course corrections; increases probability of mission failure.

(2) Places limit on maximum allowable altitude for orbiters and fly-bys thereby influencing experimental capabilities.

P'_G has been viewed principally in conjunction with accidental impact of entire vehicle, involving large numbers of organisms. A value of unity may be reasonable in this case. However, in other instances, e.g. ejecta, much smaller numbers are involved.

P'_G should, more properly, be defined as a function of the number of organisms (up to some limiting number, say, 100). But this is too cumbersome at the level of COSPAR. It may therfore be more appropriate to exclude P'_G from COSPAR agreements and expect a conservative but realistic approach to its evaluation by the implementing nations, similar to that which must be done for other parameters, e.g. P_R, N_o, D, etc.

VENUS '67 CALCULATIONS

- ALL SOURCES OF CONTAMINATION, EXCEPT ACCIDENTAL IMPACT, ARE NEGLIGIBLE FOR $P' \leqslant 3 \times 10^{-5}$

$$P' \cong \underbrace{P_{I/i} \ (q_r + q_1)}_{(P'_T)_{impact}} \cdot P'_R \cdot P'_G$$

$P_{I/i}$ - probability of achieving impact trajectory at injection

q_r - failure probability of Agena retro-rocket $\approx 10^{-2}$

q_1 - failure probability of first mid-course maneuver $\approx 2 \times 10^{-2}$

$2 \times 10^{-2} \lessdot (P_{I/i})$ unbiased $\lessdot 8 \times 10^{-2}$; $q_r + q_1 \approx 3 \times 10^{-2}$; $P'_R = 1.$

- For $P'_G = 0.1$ and $P' = 3 \times 10^{-5}$ trajectory biasing is required to reduce $P_{I/i}$.

- Trajectory bias would not be required if, for example, $P_c = 3 \times 10^{-5}$ and $P'_G \approx 10^{-2}$

or if $P_c = 3 \times 10^{-4}$ and $P'_G \approx 10^{-1}$.

Calculations are from recent Quarantine Document prepared for launch of Mariner-Venus '67.

Analysis indicates that for this mission only the parameters shown for (P'_T) impact are significants.

Generalization from this example is that the one order of magnitude range in COSPAR parameters has significant impact on implementation. The values of P_c, P'_G, and retention of maximum flexibility in allocation between P and P' are important to orbiters and fly-bys.

COST - EFFECTIVENESS (SYSTEM ANALYSIS) IN PLANETARY QUARANTINE

1. OPTIMIZE ENGINEERING SUCCESS AND NON-CONTAMINATION PROBABILITIES WITHIN ESTIMATED RESOURCE AVAILABILITY

   OR

2. MINIMIZE COST OF RESOURCES FOR DESIRED OBJECTIVES OF ENGINEERING SUCCESS AND NON-CONTAMINATION PROBABILITIES.

RELATION TO COSPAR AGREEMENTS :

1. OMISSION OF P_G AND P'_G FROM COSPAR AGREEMENTS LIKELY TO ENHANCE PROGRAM COST - EFFECTIVENESS.

2. FOR CONVENIENCE, P_c MIGHT BE DEFINED ON PER-NATION BASIS AND VALUE OF $n_L + n_U$ MADE CONSISTENT WITH OUR OWN ESTIMATES OF MAXIMUM NUMBER OF MISSIONS.

Cost effectiveness is becoming a predominant tone of discussion on planetary quarantine, although not necessarily referred to by this name, e.g. Karth hearings.

Basic ingredients in cost-effectiveness (system analysis) assessment of the problems are (1) cost of attaining various levels of non-contamination probabilities, (2) cost of attaining various levels of engineering success probabilities, (3) quantification of the effect of quarantine requirements on the cost of achieving any level of engineering success probability, (4) definition of a number of meaningful implementation approaches and (5) selection of a preferred approach within the context of limited program resources.

Whether cost-effectiveness is approached in a formal, analytical sense or in a qualitative manner, as is presently the case, it is desirable to minimize restrictive effects of COSPAR agreements on the definition of alternative approaches. Omission of P_G and P'_G can serve this purpose.

SUMMARY OF PLANNED AND PROJECTED U.S. MISSIONS WITH POTENTIAL FOR PLANETARY CONTAMINATION

PLANET	BIOLOGICAL INTEREST	PERIOD OF UNMANNED EXPLORATION	NUMBER OF LAUNCH OPPORTUNITIES DURING PERIOD	MAXIMUM ESTIMATED U.S. MISSIONS DURING PERIOD	
				n_L - Landers	n_U - Non-Landers*
MARS	YES	1964 - 1986	11	16	20
VENUS	YES	1963 - 1990	16	18	20
JUPITER	YES	1973 - 2000 ?	25 ?	1+	15+
SATURN	?	1977 - 2010 ?	34 ?	0+	5+
MERCURY	?	1973 - 2020 ?	19 ?	0+	6+
URANUS	?	1977 - ---	30 ?	0+	5+
NEPTUNE	?	1977 - ---	30 ?	0+	5+

* INCLUDING BUS FOR LANDERS

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Data derived from OSSA prospectus of 3/22/67.

Pertinent points:

(1) A value of 50 for the maximum number of U.S. vehicles in Mars mission ($n_L + n_U$) is reasonable and conservative.

(2) COSPAR will have to consider not just Mars and Venus but all other potential planetary missions. For each of these it must establish (1) biological interest and (2) the estimated period of unmanned exploration and/or number of missions involved. The addition of P_G and P'_G for all these cases would make the COSPAR task too cumbersome.

ALTERNATE	PARAMETERS SUBJECT TO COSPAR AGREEMENT				IMPLICATIONS ON OBJECTIVES:		
	P_c or P_c (U.S.A)	$n_L + n_U$	N_L and n_U	P_G and P'_G	How definitive is commitment?	How likely is it to interfere?	How does it facilitate demonstration of adherence?
A	X				POSSIBLE ALTERNATIVE		Use an agreed upon model as basic framework for demonstrating adherence.
B	X	X			Recommended Approach: Specification of $n_L + n_U$ adds meaning to P_c without affecting allocation freedom for P and P'		
C	X		X		Not Recommended: Separate definition of n_L and n_U reduces allocation flexibility without adding to definitiveness of commitment		
D	X			X	Not Recommended: (1) P_G and P'_G are not readily defined both in a simple as well as realistic manner. (2) P_G and P'_G are subject to extensive experimental evaluation (3) Uncertainty, and need for a conservative estimation approach, are about the same for P_G and P'_G as they are for P_R, P'_R, N_o, and D.		
E	X	X		X			
F	X		X	X			

For recommended approach (Alternate B), could use either :

$$P_c = 10^{-3} \qquad n_L + n_U = 100$$

or

$$P_c \text{ (U.S.A.)} = 5 \times 10^{-4} \qquad n_L + n_U = 50 \text{ (for U.S.A.)}$$

based on

$$P_c = P_c\text{(U.S.A.)} + P_c\text{(U.S.S.R.)} = 10^{-3}$$

A SIMPLIFIED MODEL

(1) $$P_c = N P_m$$

N - number of missions (a mission can consist of either a fly-by, a lander and its bus, two landers with one bus, etc.)

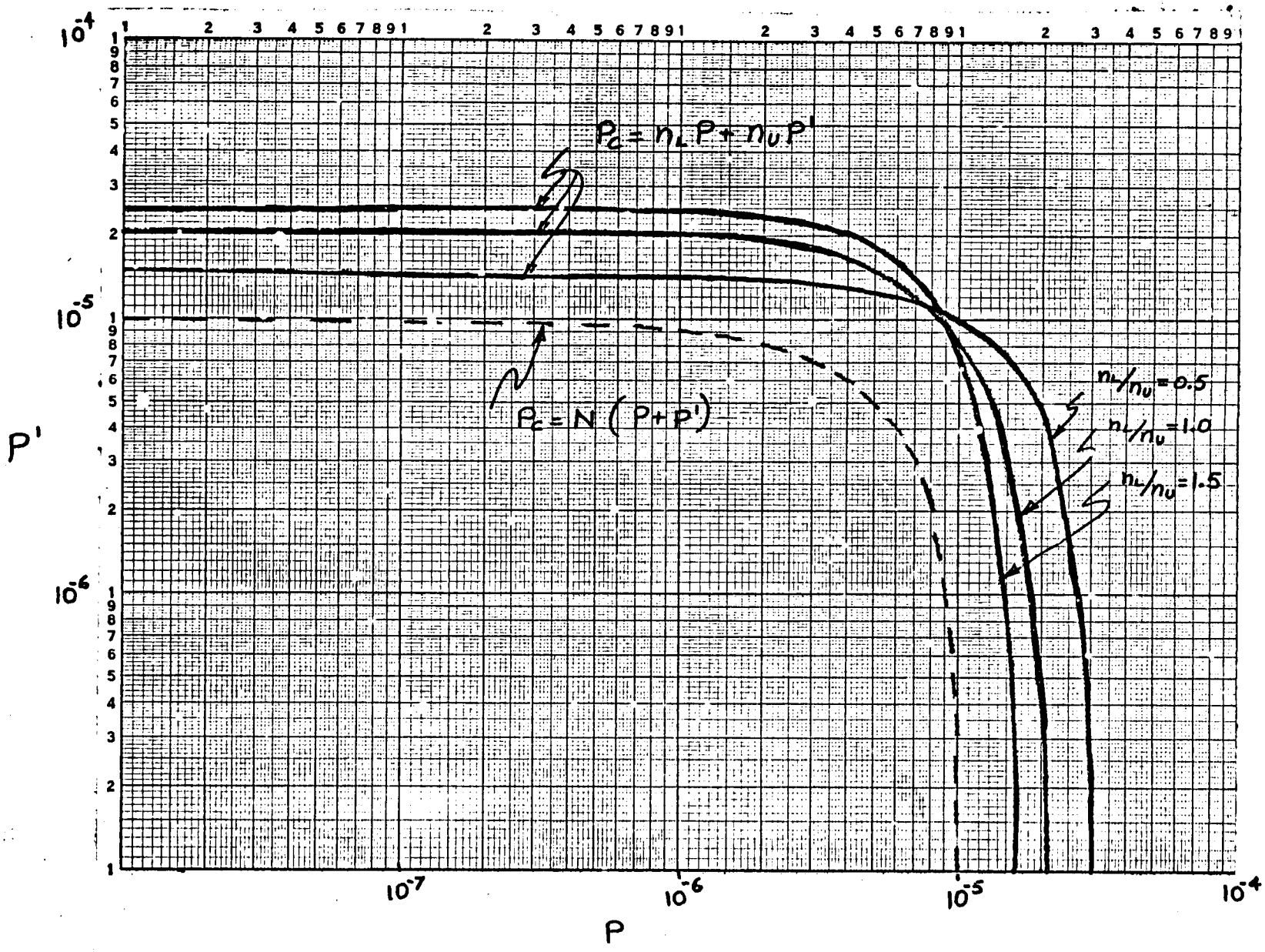
P_m - probability that a mission will contaminate planet

(2) $$P_m = \sum^{i} (P_T)_i \ (P_R)_i \ (P_G)_i$$

The i sources of contamination to be considered for a mission combines all sources of lander and bus comprising the mission.

Equations (1) and (2) offer a simple, compact model for COSPAR discussions and demonstration of adherence.

Possible disadvantage is its affect on allocation of risks between P and P'.



COMPARISON OF ALLOCATION FLEXIBILITY

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If $n_L + n_U$ is the same as N, then simplified model gives less freedom for favoring P' at the expense of P, or vice versa. The difference is by a factor of about 2.5, which may be significant for orbiter and fly-by missions.

Simplified approach could be used if it can be argued that the number of missions, N, is smaller than the sum of landers, buses, and non-landing vehicles. Prospectus chart (QR-18) could justify a value of $N \approx 11$ for U.S. Mars missions. A total of N = 30 for all launching nations may therefore be realistic, making the simplified model usable.

SAGAN - COLEMAN MODEL

$$\ln \frac{1}{p} = \frac{N/x}{P_e \cdot P_t \cdot P_l} \sigma \cdot P_m + n P_i$$

(P_c = n_L, $P_N P_R$, P_G, $n_U (P'_T)_{impact}$)

N -desired number of successfully completed experiments in unmanned Mars exploration program

x -mean number of experiments per lander

P_e -mean probability that an experiment will work as designed

P_t -probability that the lander vehicle will perform its engineering functions after it is landed on the planetary surface

P_l -probability of finding experimental conditions on Mars (e.g. kind of life) compatible with experiment design

σ -probability of one viable micro-organism on surface of Mars due to a single lander

P_m -probability that one organism deposited on the surface of Mars will survive, grow and spread, thus leading to planetary contamination

n -number of fly-bys and orbiters

P_i -probability of accidental impact by a fly-by or orbiter

1966 COSPAR EQUIVALENT

(1) $P_c = 1 - P$

$$\ln \frac{1}{p} = \ln\left(\frac{1}{1-P_c}\right) = P_c + \frac{1}{2}P_c^2 + \frac{1}{3}P_c^3 \cdots \approx P_c$$

(a) $\ln \frac{1}{p} = P_c$

Let R - probability of landing success

$$n_U \cdot R = \frac{N/x}{P_e \cdot P_t \cdot P_l}$$

Let $R \approx 1$

(b) $\frac{N/x}{P_e \cdot P_t \cdot P_l} = n_U$

(c) $\sigma = P_N \cdot P_R$

(d) $P_m = P_G$

(e) $n = n_U$

(f) only case considered is impact probability, letting $P'_R = 1$; $P'_G = 1$

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APPENDIX C

RECOMMENDED BASIC NOMENCLATURE FOR PLANETARY QUARANTINE

1. SCOPE

The prevention of contamination of planetary exploration programs entails a collaborative effort on the part of many nations and diverse technological disciplines. To facilitate the process of deriving quarantine standards and for purposes of demonstrating adherence to such standards, it is desirable to achieve a measure of uniformity in the nomenclature and definitions used. The following basic nomenclature and attendant definitions are recommended in the preparation of documents intended primarily for international distribution or which may be expected to have significant international circulation.

The terminology to be defined herein is not intended to be all inclusive and it is recognized that in operational use additional terms and definitions will be required. Such additional terminology should be consistent with the approach taken herein with regard to symbol categories and format.

Further elaboration of the analytical rationale for the typical relationships included herein may be found in Attachment I hereto. (Analytical Rationale for Basic Quarantine Relationships.)

2. RECOMMENDED NOMENCLATURE

2.1 Terminology for Definition of Planetary Quarantine Standards.

Currently, consideration is being given to the unmanned exploration of a number of extra-terrestrial bodies. The following symbols, unless otherwise noted, apply to the assessment of contamination probabilities of a particular planet.

Symbol	Definition
T	Time period of unmanned biological exploration (years) during which contamination is to be prevented.
P_c	Probability that during the time period T the planet under consideration will be contaminated so as to constitute a significant detriment to the intended program of biological exploration.
P(M), P(V), P(J)	Letters in parenthesis denote the planet for which the probability P (see definition above) is defined, e.g. the letter M for Mars, V for Venus, J for Jupiter, etc.
N	Number of vehicles intended to land or impact on the planet during the time-period T.
P(N)	Average probability that any one of the N landing vehicles will cause planetary contamination.

Symbol	Definition
N'	Number of vehicles in the planetary exploration program which are not intended to land on the planet during the time-period T. This category of flight vehicles includes orbiters, flyby's and the carriers of landing vehicles.
P(N')	Average probability that any one of the N' non-landing vehicles will cause planetary contamination.

2.1.1 Illustrative Usage of Terminology for Formulations of Planetary Quarantine Standards.

The following typical relationships illustrate the usage of nomenclature in section 2.1:

$$P_c = N\,P(N) + N'\,P(N') \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 2.1$$

Equation 2.1 is an approximation of standard probability relationship based upon the fact that in the present context P is much less than unity and that, necessarily, P(N), P(N') are smaller than P. Also, as noted in the definitions, probabilities of contamination due to a flight vehicle are averages for the category of vehicles under consideration.

2.2 Terminology for Events Leading to Planetary Contamination

Events will be denoted by lower case letters and the probability of their occurrence by the letter P followed by the event symbol in parenthesis.

When a distinction is to be made between micro-organisms which have undergone a sterilization process intended to render them non-viable, a prime over the symbol will denote the case where the micro-organisms have not been subjected to sterilization.

In the definitions which follow, the word "viable" will denote latent as well as immediate capacity for multiplication during the period of biological exploration. Also, "growth and spreading" on the planet surface or in its atmosphere will be viewed as equivalent to planetary contamination if it occurs to an extent that it becomes a detriment to biological exploration of the planet during the time-period T.

Symbol	Definition
n	Number of viable micro-organisms present.
n_o	Initial population of viable micro-organisms, e.g. at initiation of a sterilization process.
$P(n \geq 1)$ $P(n=1)$	Probability that one or more, or exactly one viable micro-organism will be present.

Symbol	Definitions
h	Transfer of viable micro-organisms to the planet so as to create a contamination hazard. Note: This is a generalized symbol for all contamination events to be considered for a flight vehicle, each event requiring assessment of probabilities of release and growth and spreading.
P(h)	Probability that the event h will occur.
r	Release onto the planet surface or into its atmosphere of organisms which have survived sterilization, given that they have been transferred to the planet.
P(r)	Probability that the event r will occur. Note: P(r) is the conditional probability of release, given that the event h has occurred.
r'	Release onto the planet surface or into its atmosphere of organism(s) not subjected to sterilization, given that they have been transferred to the planet.
P(r')	Probability that the event r' will occur. Note: P(r') is the conditional probability of release, given that the event h' has occurred.
g	Growth and spreading on the planet surface or in its atmosphere of terrestrial micro-organisms which survived a sterilization process, given that events h and r have occurred.
P(g)	Probability that the event g will occur. Note: P(g) the conditional probability of growth and spreading, given that events h and r have occurred.
g'	Growth and spreading on the planet surface or in its atmosphere of terrestrial micro-organisms which had not been subjected to sterilization, given that events h' and r' have occurred.
P(g')	Probability that the event g' will occur. Note: P(g') is a conditional probability of growth and spreading, given that events h' and r' have occurred.

2.2.1 Illustrative Usage of Terminology for Events Leading to Planetary Contamination

The following illustrates the usage of terminology in section 2.2 for the evaluation of P(N) and P(N') in Equation 2.1.

a. Probability that a lander will contaminate the planet.

Complexity of planetary landing vehicles may lead to a distinction between contamination located in different regions of the spacecraft, each requiring separate consideration of the events contributing to the total probability of contamination by the lander. A suitable frame-work for this purpose is provided by the equation

$$P(N) = \sum_{j=1}^{j=m} \left[P(h) \quad P(r) \quad P(g) \right]_j \qquad 2.2$$

provided the probability of landing is taken as unity.

As a further illustration of Equation 2.2, assume that contamination is segregated into three categories: (1) buried, or internal to materials and components (to be denoted by the subscript b), (2) occluded between mating surfaces (to be denoted by the subscript m) and (3) contamination on open surfaces (to be denoted by the subscript s). Three independent contamination events would thus be considered (m=3) corresponding to the above. Equation 2.2 can then be expressed explicity as

$$P(N) = P(n \geq 1)_b \cdot P(r)_b \cdot P(g)_b + P(n \geq 1)_m \cdot P(r)_m \cdot P(g)_m + P(n \geq 1)_s \cdot P(r)_s \cdot P(g)_s \qquad 2.3$$

Thus, P(h) is, in each case, the probability of a viable organism remaining after sterilization in the three regions considered. Different probabilities of release from buried, mated and surface contamination are possible in this formulation and, if appropriate, a similar distinction can be made for probabilities of growth and spreading.

b. Probability that an unsterilized vehicle will contaminate the planet.

Recognizing that planetary contamination may be due to a number of contamination sources, P(N') may be evaluated from

$$P(N') = \sum_{i=1}^{i=k} \left[P(h') \cdot P(r') \cdot P(g') \right]_i \quad \ldots\ldots \quad 2.4$$

where k is the total number of i independent contamination sources. (Sources which are not independent would be viewed as jointly constituting a single source.)

APPENDIX D

ESTIMATION OF MICROBIAL SURVIVAL IN HEAT STERILIZATION*

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ABSTRACT

An analytical model is developed for the survival times of a microbial population during heat sterilization in which influences attributable to the physical characteristics of the environment are distinguished from effects of exposure time. Experimental data are examined relative to this model and it is concluded that the proposed analytical approach is useful in correlating laboratory data and also in defining more realistic sterilization process requirements for spacecraft applications. However, further validation and elaboration of the model is needed in conjunction with more complete laboratory data than is currently being generated.

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**Consultant, Westat Research Incorporated

I. INTRODUCTION

Laboratory evaluations of microbial survival under various destructive environments have been studied for well over half a century. Throughout this time period, including the present, experimental data have been in conflict with the most prevalent model for microbial survival, viz. that the viable population of micro-organisms exposed to a particular sterilizing environment decreases in number by one decade in equal times of exposure (1), (2), (3). This model requires that a plot of the logarithm of survivors vs. exposure time be a straight line (4). It can be expressed analytically as

$$\frac{N(t)}{N_o} = e^{-t/D} \tag{1}$$

where N_o is the initial viable population, N (t) is the number of survivors at exposure time t, and D is a resistance parameter for the particular species and sterilization environment.

The above logarithmic model is convenient because of its analytical simplicity and, for many sterilization processes, it may well be adequate. In the case of spacecraft sterilization, however, it is necessary to account for a variety of process parameters and different--and to some degree controllable, environments. Furthermore, use of excessive safety margins in the sterilization process, due to uncertainty in the model by which requirements are specified, are undesirable because of likely detrimental effects on equipment performance. Simplicity and familiarity with the logarithnic model are therefore no longer sufficient to justify its use and lack of correlation with experimental data looms larger.

This paper describes the evolution of microbial survival models aimed at overcoming known deficiencies of the logarithmic model. The basic framework of investigation may be defined by noting that $N(t)/N_o$ in Equation (1) represents the probability that a micro-organism of a given species and subjected to a fixed sterilization environment will survive to time t. This probability of survival will be denoted as $P_s(t)$. The probability of death in the interval of time t, $P_d(t)$, is then

$$P_d(t) = 1 - P_s(t) \tag{2}$$

Underlying the probability of death $P_d(t)$ is a frequency of death, $f(\tau)$, such that

$$P_d(t) = \int_o^t f(\tau)\, d\tau \tag{3}$$

where $f(\tau)\,d\tau$ denotes the probability that a micro-organism will be rendered non-viable in the time interval between τ and $\tau + d\tau$ (5). It is readily shown that the following expression holds for the logarithmic model:

$$f(\tau) = \frac{1}{D}\ e^{-t/D} \qquad (4)$$

In the present development, frequency of death distributions other than Equation (4) are investigated. In particular, we seek distributions which can be derived from physical assumptions of the sterilization process so as to permit a meaningful interpretation of analytical and experimental results.

II ANALYTICAL MODEL DEVELOPMENT

Notably absent in the development of the logarithmic model is any allowance for the fact that a sterilizing environment may have an effect on the exposed micro-organisms which is cumulative with time. To incorporate such an effect it is convenient to rank the population of N_0 initially viable organisms in the order of their "deaths" during sterilization. Thus, let τ_j denote the time of death of the j-th organism, τ_{j-1} the time of death of the ^{j} immediately preceding organism, etc. It will be assumed that a single species is involved and that the death of one organism is independent of the death of any other organism within the population. For analytical purposes attention is focused on the incremental time $\tau_j - \tau_{j-1}$, i.e. the additional time which will elapse before the j-th organism dies, ^{j} $^{j-1}$ given that the preceding one has just died. If prior exposure time is a major factor, then the incremental time would be a function of τ_{j-1}. However, other factors are, clearly, also present.

In general, the destruction of micro-organisms in a sterilization process can be categorized from two points of view. On the one hand, a distinction can be made between random and constant phenomena. For example, a population of micro-organisms can be viewed as having a probabilistic distribution of life-times, whereas the temperature of sterilization might be considered constant, i.e. only the average value of random molecular motion would be viewed as being relevant. On the other hand, a distinction can also be made between parameters associated with the micro-organisms and those of their physical environment, e.g. the physical characteristics of the medium in which they are located. The process of destruction clearly involves an interaction between the micro-organisms and the physical environment and it would be desirable to establish whether randomness is a characteristic of only the micro-organisms, of the destructive process acting through the environment, or of both. This question is left to physical modeling which must complement analysis and experimentation. For the present, the analytical models to be considered will allow for the existence of a random variable, ϵ_j, as well as non-random variables,

without explicitly associating them with the environment or the micro-organisms. Thus, ϵ_j will represent the magnitude which the random variable assumes during the interval between τ_{j-1} and τ_j.

The first hypothesis to be considered focuses on the cumulative influence of exposure time. Specifically, it is assumed that the incremental death time is directly proportional to prior exposure time, viz.

$$\tau_j - \tau_{j-1} = \tau_{j-1} \epsilon_j \quad \text{for} \quad j = 2, 3, 4, \ldots k, \ldots N \tag{5}$$

To obtain a continuous relationship for the probability of death, Equation (5) is rearranged and both sides summed over the time interval t_k. Thus,

$$\sum_{j=2}^{j=k} \frac{\tau_j - \tau_{j-1}}{\tau_{j-1}} = \sum_{j=2}^{j=k} \epsilon_j \tag{6}$$

The summation starts at j=2 since τ_o is undefined. Strictly speaking, therefore, this model accounts only for death times beyond τ_1, but this is hardly a serious limitation.

The left hand side of (6) can be approximated by an integral if N_o and t_k are large. This is generally the case and we can write

$$\sum_{j=2}^{j=k} \frac{\tau_j - \tau_{j-1}}{\tau_{j-1}} \approx \int_{t_1}^{t_k} \frac{d\tau}{\tau} = \ln t_k - \ln t_1 \tag{7}$$

It will be convenient to carry the $\ln t_1$ term to the right side of (6) and view it as a random variable, ϵ_1 independent of $\epsilon_2, \epsilon_3, \ldots, \epsilon_k$. Equation (6) and (7) thus yield

$$\ln t_k = \sum_{j=1}^{j=k} \epsilon_j \tag{8}$$

The summation of ϵ_j in Equation (8) can be evaluated by applying the central limit theorem (14). Thus, under the general regularity conditions of this theorem, and with k sufficiently large, it follows that the summation, and hence $\ln t_k$, is normally distributed with a mean value, μ' and variance σ'^2. The implication of this result is that if exposure time has a cummulative effect, and if this effect has the simple relationship of Equation (5), then the logarithm of survival times would follow a normal distribution (6). Specifically, the probability of

survival would be expressed as (7)

$$P_s(t) = \frac{N(t)}{N_o} = 1 - \frac{1}{\sqrt{2\pi}} \cdot \frac{1}{\sigma'} \int_0^t \exp\left[-\frac{1}{2\sigma^2}(\ln\tau - \mu')^2\right] d \ln(\tau) \quad (9)$$

(The subscript k in t_k has been supressed since its use is restricted to the derivation and is not relevant to the final result above.)

The shapes which the log-normal model of Equation (9) produce for semi-log plots of $N(t)/N_o$ versus exposure time are shown in Figure 1. These shapes are not unlike the ones observed for experimental data, nor does this model exclude the essentially straight lines noted for some experimental data.

It is also of interest to consider a second hypothesis. Specifically, assume that prior exposure time does not produce a cumulative effect in the destruction process, i.e. that only the environment and the random process in it, or in the micro-organisms, determine the distribution of survival times. This hypothesis can be formalized by writing

$$\tau_j - \tau_{j-1} = \epsilon_j \quad (10)$$

Following a procedure identical to that used for Equation (5), it is readily shown that, in this case, the survival time t, rather than the logarithm of t, would be normally distributed with mean value μ'' and variance $(\sigma'')^2$.

Finally, both hypothesis can be combined into one model so as to allow for the simultaneous existence of environmental and time-cumulative effects. Thus, let

$$\tau_j - \tau_{j-1} = (a\,\tau_{j-1} + b)\,\epsilon_j\,, \quad (11)$$

where a and b are parameters denoting the relative influence of time-cumulative and environmental effects, respectively. It is evident from Equation (11) that during the initial time of sterilization, when $b >> a\,\tau_{j-1}$, the environmental parameters would dominate and the previously derived normal distribution of survival times would be prevalent. Similarily, for large values of exposure time, when $a \cdot \tau_{j-1} >> b$, the distribution of survival times to be expected from this model would be lognormal, i.e. a normal distribution of the logarithms of exposure times. The general distribution, applicable over the entire range of times, which follows from the hypothesis of Equation (11) can be expressed as a normal distribution of $\ln\left(\frac{t + c}{c}\right)$, where $c = b/a$.

in viable population during the early phase of heat application (8), (9).)

Figure 3 is typical of other experimental data studied in which the spores are placed on paper strips and temperature is the parameter varied in the experiments. Again, a deviation from log-normality is noted for the initial heating times. However, the extent of this deviation - in terms of the value of N/N_o beyond which the log-normal model applies, is small and does not seem to depend upon temperature. Constancy of the slope in the two sets of data is again noted.

In the experiments of Figure 4 the spores were encapsulated in Lucite (8), (9), and the environmental parameter, b, is seen to dominate for a significant portion of time. Thus, about three decades of reduction take place at all three temperatures before time-cumulative effects produce log-normal behavior, i.e. when $a \cdot t$ becomes much larger than b.

Figure 5 has been included to illustrate the potential utility of this model in identifying relevant experimental conditions. Thus, the data of Figure 5 is reported tc have been obtained under identical experimental procedures (10) as that of Figure 4 so as to add three more temperatures to those tested in Figure 4. It is noted, however, that the slopes of the log-normal lines in Figure 5, although the same for this set of data, are significantly different than in Figure 5. Thus, in Figure 4 $\sigma = 0.57$ whereas in Figure 5 $\sigma = 0.305$. Based on the present model, it can be speculated that the spore populations used in these two sets of experiments, which were carried out a few months apart, were not identical.

All of the above comparisons focus on the late portion of the survival curves where log-normal behavior is anticipated. Based on the model described herein deviations from log-normality would be expected during initial heating and are observed. However, the model also requires that during the initial portion of the curve, when environmental effects are dominant, survival time, t, should be normally distributed. This has been observed by Wax (11), and further support is also indicated in Figure 6. In this Figure the survival data of Figure 4 is plotted on normal probability paper and a reasonably good fit to a straight line is obtained.

Figure 7 provides a log-normal plot of data showing the effect of water activity on the survival of spores encapsulated in Lucite (12). In the context of the present model, water activity would obviously be viewed as an environmental parameter and associated with b. Water activity would therefore be expected to shift the knee of the curve on a log-normal plot, as was the case for the environmental effects of Figure 2. This shift is evidenced in Figure 7.

To consolidate the analytical development, we will associate a mean value μ and variance σ^2 with the hypothesis of Equation (11). The three cases of interest can then be summarized as follows:

Condition	Variable Normally Distributed	Distribution Mean	Distribution Variance
General	$\ln\left(\frac{t+c}{c}\right)$	$a\mu$	$a^2\sigma^2$
$at \ll b$	t	$b\mu$	$b^2\sigma^2$
$at \gg b$	$\ln t$	$a\mu$	$a^2\sigma^2$

$c = b/a$ t - survival time

III COMPARISON WITH EXPERIMENTAL DATA

Laboratory data provides information on the probability of survival, N/N_o, for various heating times, t, and a plot of such data on normal probability paper, either against t or lnt, should therefore provide a test of the validity of the model. Since evolution of the model started with the log-normal case (6), much of the data analysis was in terms of this limiting case. Indeed, deviations from the log-normal model noted in these early analyses (6) have led to the more general model described herein. The following discussion of experimental data will therefore follow a similar pattern. Thus, conformance to the log-normal model will be tested first with the expectation, however, that it will only be valid for long heating times. Verification will then be sought separately for a normal distribution of survival times during initial heating.

Figure 2 is a plot of typical heat-survival data on log-normal paper, i. e. the graph paper is so constructed that if microbial survival follows a log-normal model, a straight line should result. Furthermore, the intersection of this line with the time axis will permit calculation of the mean $a\mu$ and its slope will yield the variance $a^2\sigma^2$. Figure 2 shows experimental data for the same spore species subjected to heat sterilization in three different environments, viz. in air, helium and vacuum. For long heating times, a straight line through the data appears to be justified, suggesting compliance with the model. Furthermore, it is not unreasonable to use the same slope for the three sets of data. This implies that the variance did not change as the environment was altered. Hence, the variance might be attributed to characteristics of the spore population used in all three experiments. Referring to Figure 2 again, deviation from log-normality during initial heating is evident in all three cases, as indicated by the dashed lines, the amount being a function of the environment. (On semi-log plots these deviations would appear as a pronounced drop

IV. DISCUSSION

The preceding consideration of laboratory data was largely qualitative in that it focused on comparing the data with trends predicted by the model. More detailed computer analysis is possible, and is currently in progress, capable of extracting quantitative values for the parameters c, the mean, $a\mu$, and variance $a^2\sigma^2$. However, such analysis, to be meaningful, requires more complete laboratory data than is currently available. Specifically, the data must extend over at least six decades of population reduction and have an adequate number of points throughout this range. Given such data, it is seen from the summary relationships of Section II that the parameters of interest can be obtained in a number of independent ways thereby providing means of verification as well as methods for further testing the validity of the model.

Inadequacy of currently available data precluded establishment of a functional relationship for the temperature dependence of microbial resistance in the context of the present model. An attempt to do this was made using data such as that shown in Figure 4. Since the slope of these curves remains essentially constant as temperature is changed, it can be assumed that only the mean value varies with temperature. Plots of mean value versus temperature were therefore made to establish the form of temperature dependence (6). It was thus found that on the basis of the available data, the mean values ($a\mu$) could be proportional either to T, $T^{1/2}$, or $T^{3/2}$, where T is temperature in degrees absolute. Since these relationships cannot all be valid, it is concluded that temperature dependence may be of the form T^n. The value of the exponent n cannot be established until experimental data is available over a broader range of temperatures. However, even the above attempt has some utility as it showed that the Arrhenius form of temperature dependence, i.e. where the mean value would be proportional to $e^{k/T}$, is not consistent with the present model even for the limited data now available.

It will be recalled that the hypothesis underlying the time-cumulative part of the model states that incremental survival time is directly proportional to prior exposure time, i.e. the longer the spore has been exposed, the greater the probability of its surviving further. A "tailing off" is therefore an integral characteristic of survival curves predicted by the model.

It is tempting to compare sterilization process times required by the present and the logarithmic (D-value) models so as to gauge the added assurance of sterility provided by the model considered herein. Such a comparison is only of limited value because of the arbitrariness which is necessarily associated with the assignment of a D-value to data which generally does not produce a straight line on a semi-log plot. In those instances where a straight line could reasonably be assumed, the present model would suggest the absence of dominant environmental effects, permitting use of the limiting case of a log-normal distribution. Under these conditions, Figure 1 (a)

can be utilized by assuming that laboratory data extends over about five decades of population reduction and that the dashed lines represent the straight (D-value) lines which would be drawn from the data. It is seen from Figure 1 (a) that, if process time were based on a required ten decades of population reduction, the log-normal model would call for approximately twice the sterilization time of the D-value model. Generally, however, environmental effects are present and their inclusion in the present model would bring the relative sterilization times closer together. More significantly, use of the present model would lead to a more rational basis for selecting safety factors for sterility assurance and for relating these safety factors to the explicit conditions on which process requirements are to be based.

V. SUMMARY AND CONCLUSIONS

The analytical model described herein formalizes the hypothesis that heat inactivation of micro-organisms is due to distinct environmental and time-cumulative effects interacting with a stochastic process. Analysis of laboratory data in the context of this model offers support for the distinction between the above two effects and indicates the utility of the present model as a framework for further development as well as for immediate use. Environmental effects, represented by the parameter b in the model, appear to be largely associated with characteristics of the medium in which the organisms are located, including, but not limited to, its moisture content. To identify environmental influences, it is thus necessary to observe behavior of the survival curve during the initial phase of heating as well as during transition to the later log-normal phase (when time-cumulative effects predomiate). Although adequate laboratory data focusing on the initial phase is not as yet available, investigations to-date suggest a normal distribution for the probability of survival with exposure time when environmental effects are the dominant mechanism of inactivation. On a relative basis, more experimental data is available in support of log-normal behavior for long heating times. The need to account for prior exposure time in predicting future survival probabilities is therefore reasonably well established.

From work done to-date, the following conclusions can be drawn:

(a) Heat inactivation of micro-organisms is a sufficiently complex process to require a more comprehensive analytical structure than is offered by the currently prevalent logarithmic model. To support development of such a model, it is essential that laboratory data be obtained in a manner consistent with the complexity of the process. Thus, heat inactivation experiments must be extended to at least six decades of population reduction and survival points obtained throughout this range, including the early phase of heat inactivation. Development of analytical models should

also be complemented by physical modeling and both closely related to measured parameters in laboratory experiments. Thus, recent interest in the effects of water activity on microbial destruction could be incorporated in a physical model of moisture diffusing between a spore and its surrounding medium. Clearly, such a physical model should be consistent with the analytical model, such as the one described herein, so as to lend more meaning to the underlying hypotheses and their parameters. *

(b) The model described herein can be of immediate practical use in two ways. First, it offers an improved framework for defining results of laboratory experiments and may make it possible to associate the data with distinct features of the experiment, e.g. the variance could be used to characterize the microbial species and/or the manner in which it was grown, the parameter c could define the relative influence of environmental versus time-cumulative effects in the heat inactivation process and the mean value could identify resistance at a given temperature. The model can also be used to define realistic sterilization process requirements based on parameter values derived from the log-normal phase, since process times are principally concerned with survival probabilities of the most resistant part of the population.

It cannot be claimed that the model described herein explains the mechanism of microbial inactivation by heat. However, this work is believed to be a useful step towards the development of an analytical framework essential to a realistic formulation of sterilization objectives and their implementation in the complex spacecraft characteristic of planetary exploration.

Acknowledgment

Provision of detailed experimental data, supplementary to published results, by Dr. Robert Angellotti (Taft Sanitary Engineering Center of the U. S. Public Health Service, Cincinnati, Ohio) is gratefully acknowledged. The authors also wish to acknowledge the effective support of Saul Honigstein, of Exotech Incorporated, in the data analysis and model development reported herein.

*Preliminary studies in this direction have been undertaken by M. Barrett of Exotech Incorporated.

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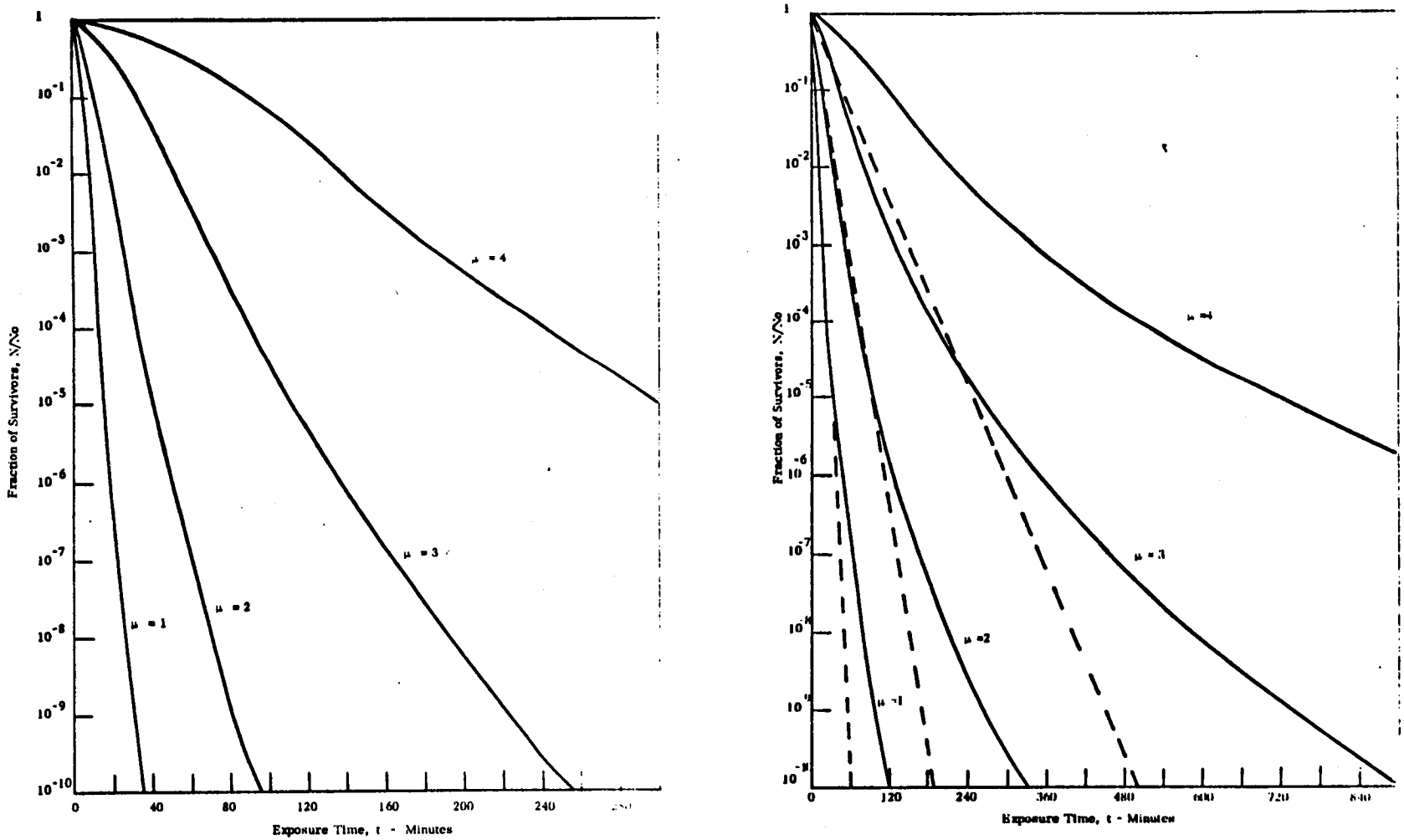


(a) σ = 0.4 (b) σ = 0.6

Fig. 1 Hypothetical Survivor Curves Based on Log-Normal Model

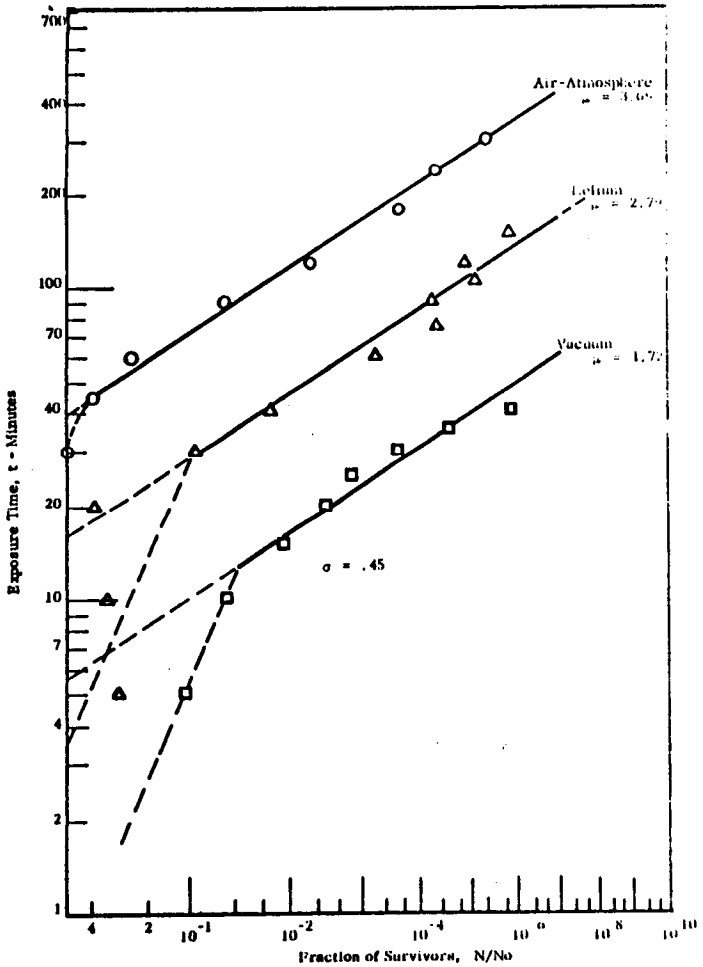


Fig. 2 Log-Normal Plot of Survival Data for BACILLUS SUBTILIS var. NIGER at 120 C

Source: Ref. 13, p. 43

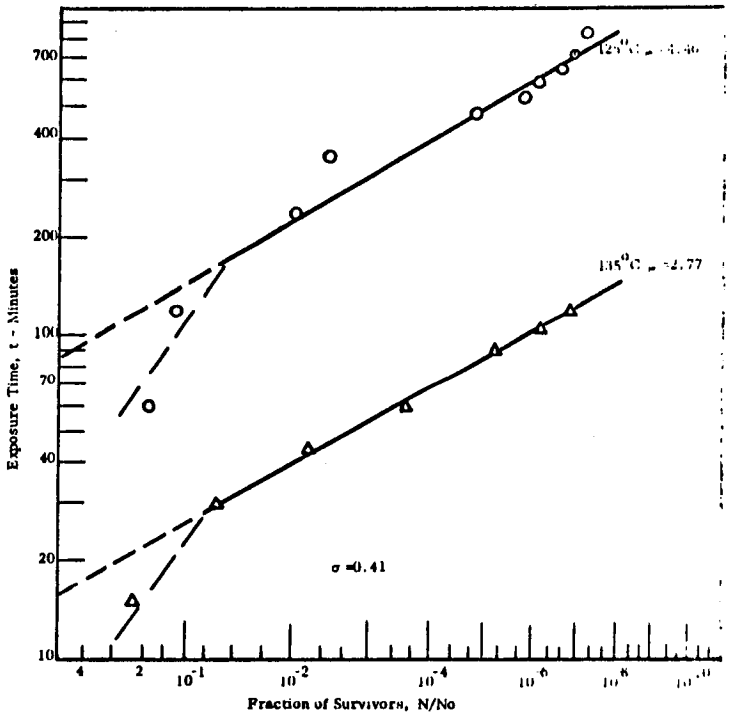


Fig. 3 Log-Normal Plot of Survival Data for BACILLUS GLOBIGII Spores on Paper Strips

Source: Ref. 8, Fig. 3; Ref. 9, Fig. 8.

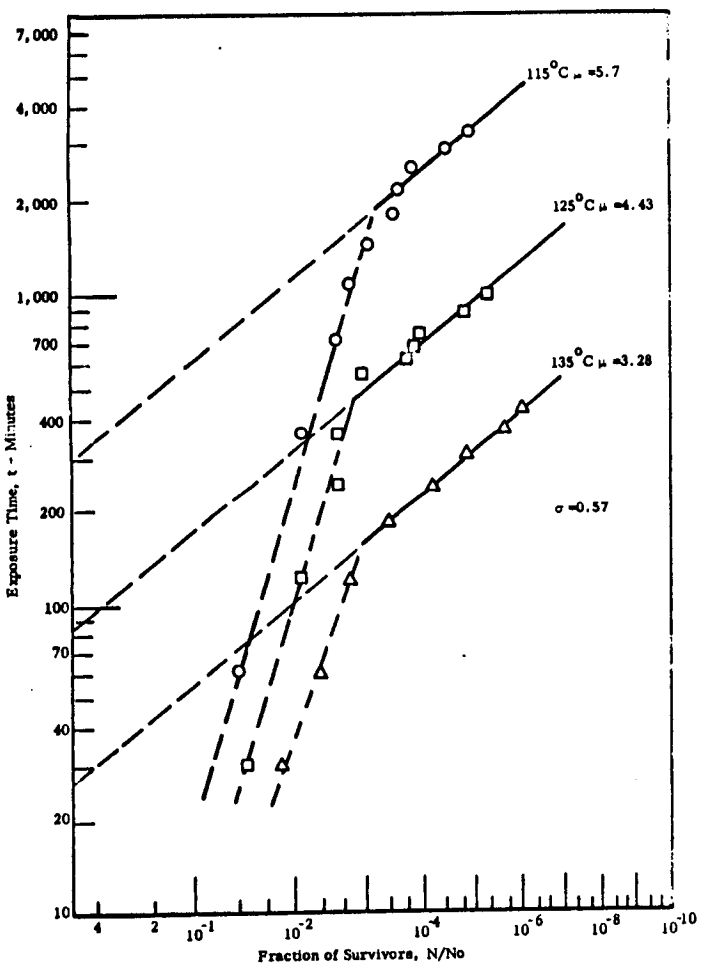


Fig. 4 Log-Normal Plot of Survival Data for BACILLUS GLOBIGII Spores in Lucite

Source: Ref. 8, Fig. 2; Ref. 9, Fig. 1, 4.

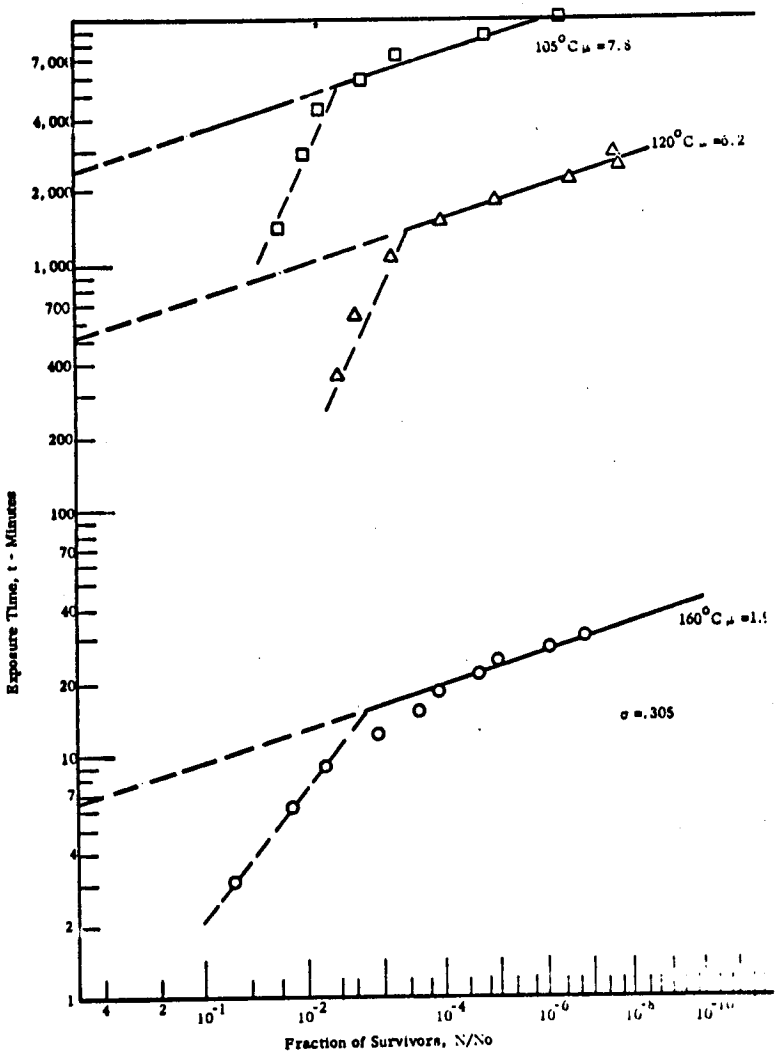


Fig. 5 Log-Normal Plot of Survival Data for BACILLUS GLOBIGII Spores in Lucite

Source: Ref. 10, and Tabulated data from Dr. Robert Angelotti.

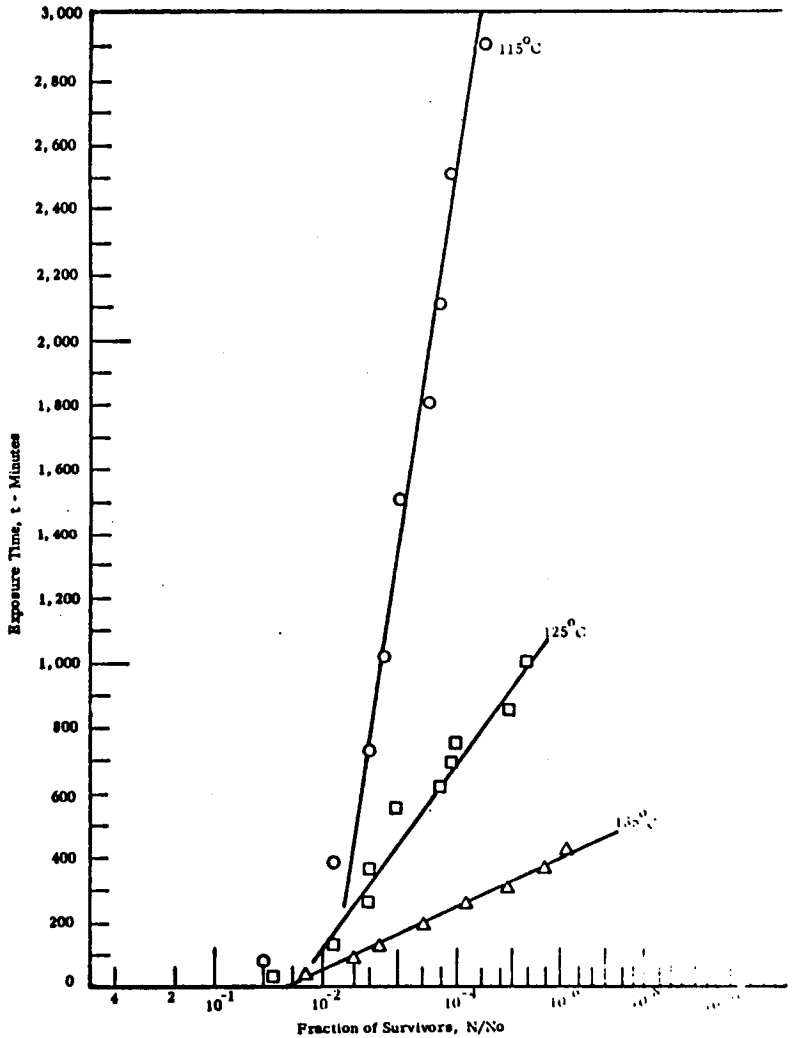


Fig. 6 Normal Plot of Survival Data for BACILLUS GLOBIGII Spores in Lucite

Source: Same as Fig. 4.

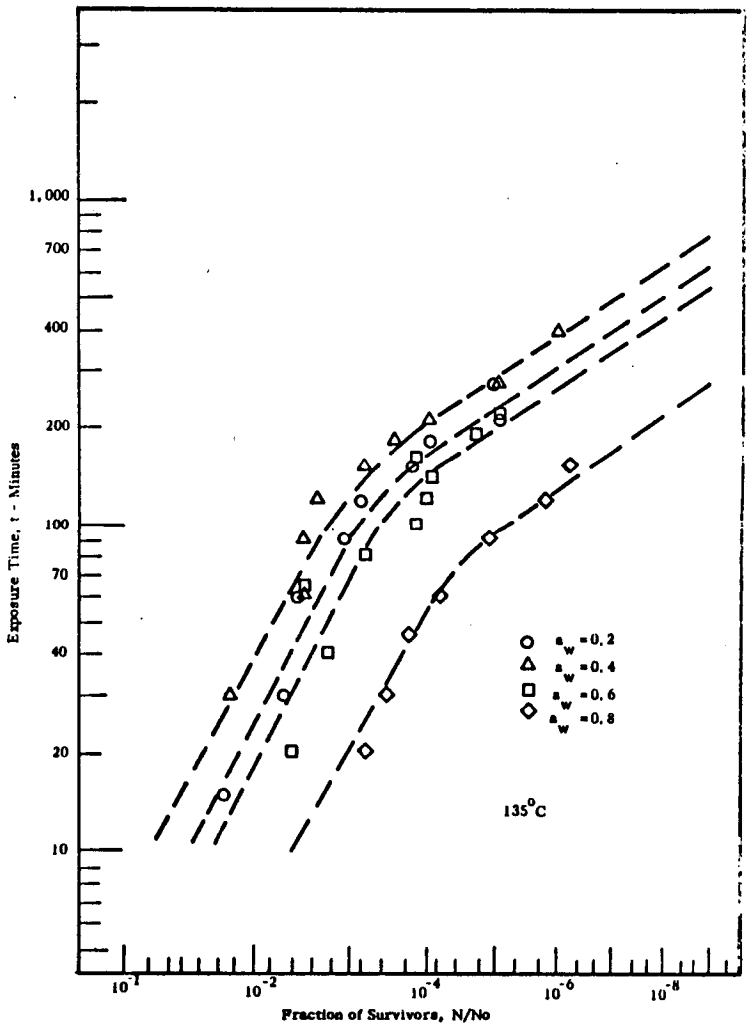


Fig. 7 Log-Normal Plot of Survival Data for BACILLUS GLOBIGII Spores in Lucite

Source: Ref. 12 and Tabulated data from Dr. Robert Angelotti.

APPENDIX E

Estimation of Log-normal Model Parameters

The purpose of this appendix is to describe the computational procedure employed to estimate the log-normal model parameters, μ, σ and c, from experimentally determined survival data.

Characterization of Experimental Data

Each determination of μ, σ and c is obtained from experimental observations of the following form:

n = Number of experimental observations

t_k = Time associated with the kth observation ($k = 1, 2, ---, n$)

$\left(\frac{N}{N_o}\right)_k$ = Observed proportion of microbial survivors at time t_k ($k = 1, 2, ---, n$)

In terms of the three-parameter log-normal survival model, and assuming unbiased measurements, the mean, or expected, value of $\left(\frac{N}{N_o}\right)_k$ is given by

$$E\left\{\left(\frac{N}{N_o}\right)_k\right\} = 1 - F\left[\alpha(t_k)\right] \qquad \text{(E-1)}$$

where

$$F(\alpha) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\alpha(t_k)} e^{-x^2/2}\, dx \qquad \text{(E-2)}$$

$$\alpha(t_k) = \frac{1}{\sigma}\left[\ell n\ \frac{t_k + c}{c} - \mu\right], \qquad \text{(E-3)}$$

$$k = 1, 2, ---, n.$$

The estimates $\hat{\mu}$, $\hat{\sigma}$ and $\hat{c}$ of the unknown parameters μ, σ and c, respectively, have been computed by minimizing the mean square deviation of $\ell n\ \left(\frac{t_k + c}{c}\right)$ from its equivalent determined by Expression (E-1) over all values $k = 1, 2, ---, n$ $\left[\left(\frac{N}{N_o}\right)_k\right.$ being substituted as an approximation of $\left.E\left\{\left(\frac{N}{N_o}\right)_k\right\}\right]$. The function which is

minimized therefore takes the form

$$Q^2 = \frac{1}{n} \sum_k \left[\ell n \left(\frac{t_k+c}{c}\right) - \sigma \; F^{-1} \left\{ 1 - \left(\frac{N}{N_o}\right)_k \right\} - \mu \right]^2 . \qquad \text{(E-4)}$$

Alternate Residual Functions

Computational ease was the primary criterion for the particular choice (Expression (E-4)) of function, Q^2, to be minimized. In this form the "residual" function, $\ell n \left(\frac{t_k+c}{c}\right) - \sigma F^{-1} - \mu$, is linear in μ and c. Therefore, for any specific value of c, Q^2 is quadratic and minimization with respect to μ and σ constitutes a non-iterative operation. (In the actual computing routine, values of c are appropriately scanned and, for each choice, Q^2 is minimized with respect to μ and σ.) A possible drawback in this approach is that, in transforming the observations $\left(\frac{N}{N_o}\right)_k$ into $\sigma F^{-1} \left[1 - \left(\frac{N}{N_o}\right)_k \right]$, deviations of $\left(\frac{N}{N_o}\right)_k$ from $E \left[\left(\frac{N}{N_o}\right)_k \right]$ may introduce undesirable biases into the parameter estimates. Further study of this possibility is warranted, including examination of alternate choices of residual functions. In this connection, a few comments are in order insofar as the more or less standard residual form

$$\left(\frac{N}{N_o}\right)_k - \left[1 - F\left\{ \alpha \left(t_k \mid \mu, \; \sigma, \; c \right) \right\} \right], \qquad \text{(E-5)}$$

i.e., the actual difference between the empirical and theoretical survival curves. Although biases of the previously mentioned type are not generated using the residual function of Expression (E-5), another potentially serious drawback is present. The attained differences between the experimental observations and the corresponding values on the theoretical curve obtained by minimizing the mean square residual, in general, increase percentage wise with increasing values of the time, t_k. This is due to the fact that $E \left[\left(\frac{N}{N_o}\right)_k \right]$ decreases substantially with increasing time and the various residual values are equally weighted. This characteristic is undesirable from the standpoint of subsequent and intended extrapolations based upon the theoretical survival curve. Rather, a related residual function of the following form would perhaps be most appropriate:

$$w_k \left[\left(\frac{N}{N_o}\right)_k - \left\{ 1 - F\left(\alpha(t_k \mid \mu, \sigma, c)\right\}\right]. \qquad \text{(E-6)}$$

$$(k = 1, 2, ---, n)$$

In Expression (E-6) the symbol w_k denotes a residual weighting function whose values can be chosen to reflect either the relative confidence in the various observations or the relative importance of the various portions of the survival curve.

The remainder of this appendix is a somewhat detailed description of the digital computing program employed in minimizing Q^2 as defined in Expression (E-1). The logical flow diagram for this program is indicated in Figure (E-1) and the following program outline relates to the components of this diagram.

Read Input

Data read into and required by the computing routine consist of the following:

n = No. of observations

t_k = Time associated with the kth observation ($k = 1, 2, ---, n$)

N_k = Observed number of survivors at time t_k

$N_k^{(o)}$ = Initial size of population associated with the kth observation

Δc_ν = νth grid size for scanning

Presets

The following quantities* are defined and computed prior to initiation of the minimization of Q^2:

$$y_k = F^{-1}\left[1 - \left(\frac{N}{N_o}\right)_k \right]; \qquad k = 1, 2, ---, n \qquad \text{(E-7)}$$

$$\bar{y} = \frac{1}{n} \sum_{k=1}^{n} y_k \qquad \text{(E-8)}$$

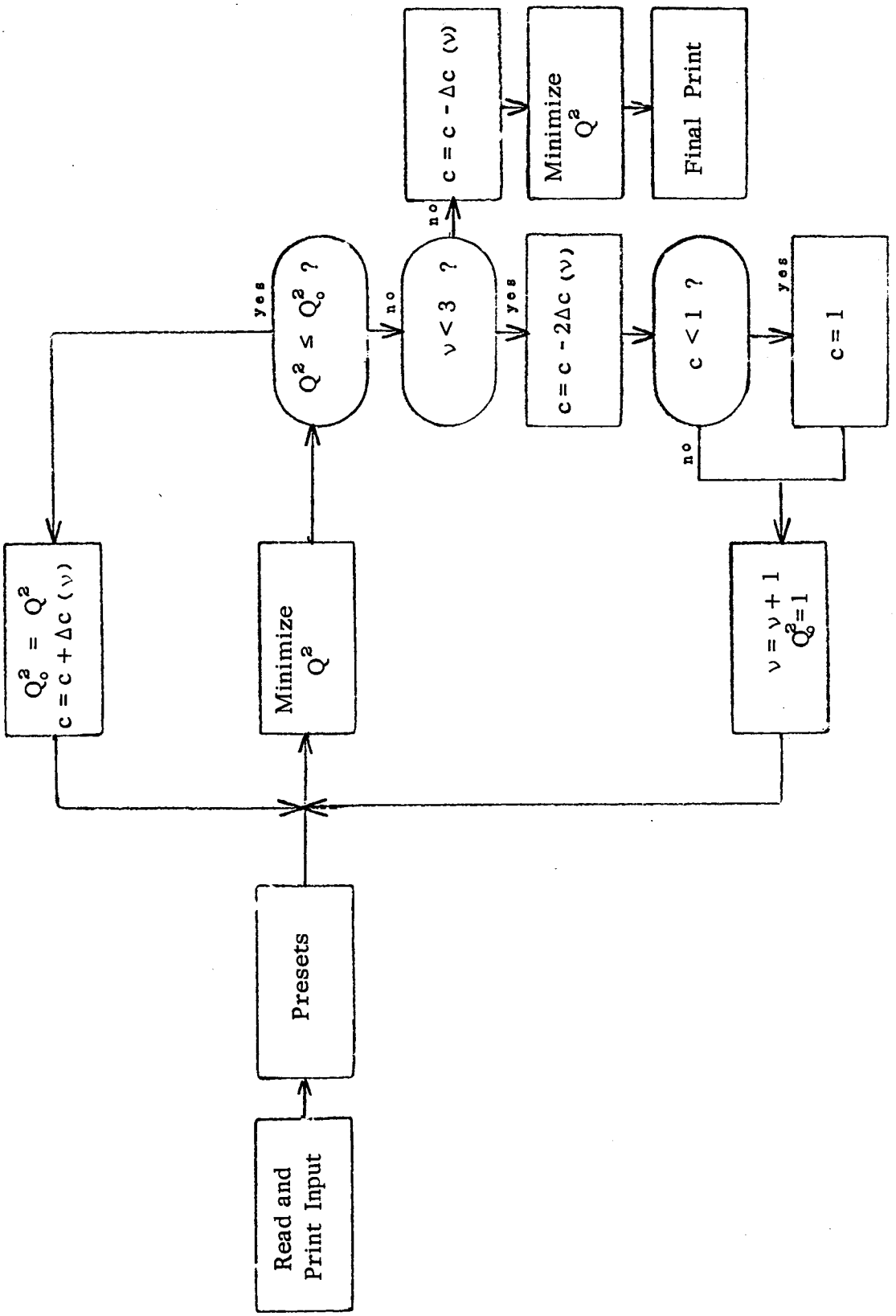
$$\overline{y^2} = \frac{1}{n} \sum_{k=1}^{n} y_k^2 \qquad \text{(E-9)}$$

$$\bar{p} = \frac{1}{n} \sum_{k=1}^{n} \left(\frac{N}{N_o}\right)_k \qquad \text{(E-10)}$$

$$v_y = \overline{y^2} - \bar{y}^2 \qquad \text{(E-11)}$$

* Presets relating to logical controls of the computing program are omitted.

FIGURE (E-1) - MAIN FLOW DIAGRAM



Minimization of Q^2

For successive fixed values of c, the following computations are accomplished:

$$x_k = \ell n \left(\frac{t_k + c}{c}\right) \; ; \quad k = 1, 2, ---, n \tag{E-12}$$

$$\bar{x} = \frac{1}{n} \sum_{k=1}^{n} x_k \tag{E-13}$$

$$\overline{xy} = \frac{1}{n} \sum_{k=1}^{n} x_k \, y_k \tag{E-14}$$

$$\mu = \frac{1}{v_y} \left[\overline{xy} - \bar{x}\,\bar{y} \right] \tag{E-15}$$

$$\sigma = \frac{1}{v_y} \left[\bar{x}\,\bar{y}^2 - \overline{xy}\,\bar{y} \right] \tag{E-16}$$

As indicated, these computations produce estimates, $\hat{\mu}$ and $\hat{\sigma}$, for the selected values of $\hat{c}$. The successive values of Q^2, determined by these estimates, are tested until essentially no improvement is noted. At this point the associated estimates of μ, σ and c are assumed to minimize Q^2.

APPENDIX F

Effects of Heat-up and Cool-down on Heat Sterilization

Introduction

The purpose of this appendix is to quantitatively formulate the effect of the heat-up and cool-down phases of heat sterilization cycles on microbial survival probabilities. To this end, the three-parameter log-normal model of microbial survival is assumed; i.e., the probability of microbial survival to time t, measured from the beginning of the sterilization cycle, is represented by

$$P_s(t) = 1 - \frac{1}{\sqrt{2\pi}} \int_0^t \frac{1}{(x+c)} \exp\left[-\frac{1}{2\sigma^2}\left\{\ell n\left(\frac{x+c}{c}\right) - \mu\right\}^2\right] dx. \qquad (F\text{-}1)$$

In this expression, μ, σ and c denote distribution parameters whose values are determined by the particular microbial population and sterilizing environment under consideration. If the sterilization cycle is taken to be the time interval during which the temperature of the environment is maintained at its maximum value, T, then the distribution parameters are assumed constant and expression (F-1) can be rewritten

$$P_s(t) = 1 - \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\alpha(t|\mu,\sigma,c)} e^{-x^2/2}\, dx \qquad (F\text{-}2)$$

where

$$\alpha(t|\mu,\sigma,c) = \frac{1}{\sigma}\left[\ell n\left(\frac{t+c}{c}\right) - \mu\right]. \qquad (F\text{-}3)$$

However, survival probabilities determined by Expression (F-2) are conservative to the extent that they ignore the effects of environmental heat-up and cool-down. These effects can be taken into account if one assumes that Expression (F-1) remains valid when the distribution parameters are appropriately varied with temperature. Since parameter variation with temperature during heat-up and cool-down implies variation with time, the integration required by Expression (F-1) is, in general, quite complicated. The development described herein, however, produces an expression for $P_s(t)$ in the form of Expression (F-2) which is applicable

to sterilization cycles which include heat-up and cool-down. In particular, for an arbitrarily specified time t and temperature - time profile $T = T(t)$, an "equivalent" time, t*, is defined such that

$$P_s(t) = 1 - \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\alpha(t^* | \mu, \sigma, c)} e^{-x^2/2} \, dx \qquad \text{(F-4)}$$

where

$$\alpha(t^* | \mu, \sigma, c) = \frac{1}{\sigma} \left[\ell n \left(\frac{t^* + c}{c} \right) - \mu \right] \qquad \text{(F-5)}$$

and where μ, σ and c are the parameter values associated with the temperature at time t.

Simplifying Assumptions

On the basis of values of μ, σ and c determined for various collections of empirical survival data, only μ and possibly c show variation with temperature. Hence, σ shall be assumed invariant with temperature.

With little or no loss of generality the temperature - time profile, $T = T(t)$, shall be taken as a "step" function, i.e., the total time interval is partitioned into n successive subintervals of length Δt_i $(i = 1, 2, ---, n)$ and $T(t)$ is assumed constant (T_i) within any one subinterval. In reality, this formulation is an approximation. However, by appropriate choices of n and the Δt_is, the approximation can be made as accurate as desired. Moreover, computational practicalities invariably require a representation of this type.

Finally, the dependence of the distribution mean, μ, on temperature is assumed to take the form

$$\mu = \mu_o + k T^m, \qquad \text{(F-6)}$$

where μ_o, k and m are constants. This expression is also based upon comparisons of collections of empirical data (1, 2).

Notation

For the purpose of the subsequent derivation, the following notational conventions are assumed:

$t_k = \sum_{i=1}^{k} \Delta t_i$ = Endpoint of the kth time interval (k = 1, 2, ---, n)

$\mu_k = \mu(T_k)$ = Value of the parameter μ associated with the kth temperature (k = 1, 2, ---, n)

$c_k = c(T_k)$ = Value of the parameter c associated with the kth temperature (k = 1, 2, ---, n)

$\tau_k = \tau(T_k)$ = Time exposed at constant temperature T_k equivalent to actual exposure to time t_{k-1}

$t_k^* = t^*(T_k)$ = Time exposed at constant temperature T_k equivalent to actual exposure to time t_k

$= \tau_k + \Delta t_k$.

Expression for t_n^*

For each value of k we shall sequentially determine t^*_k such that

$$P_s(t_k) = 1 - \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\alpha(t_k^* | \mu_k, \sigma, c_k)} e^{-x^2/2} \, dx \qquad \text{(F-7)}$$

where

$$\alpha(t_k^* | \mu_k, \sigma, c_k) = \frac{1}{\sigma} \left[\ell n \left(\frac{t_k^* + c_k}{c_k} \right) - \mu_k \right]. \qquad \text{(F-8)}$$

During the initial time interval $t_1 = \Delta t_1$ the temperature to time t_1 is constant (T_1); hence $t_1^* = \Delta t_1$.

Now, by definition of τ_2 we see that

$$\alpha(t_1^* \mid \mu_1, \sigma, c_1) = \frac{1}{\sigma} \left[\ell n \left\{ \frac{\tau_2 + c_2}{c_2} \right\} - \mu_2 \right]. \qquad \text{(F-9)}$$

Hence, for t_2^* we have

$$t_2^* = \tau_2 + \Delta t_2$$

$$= \left[e^{k(T_2^m - T_1^m)} - 1\right] c_2 + \frac{c_2}{c_1} e^{k(T_2^m - T_1^m)} \Delta t_1 + \Delta t_2 \ . \qquad \text{(F-10)}$$

Next, we see that

$$\alpha(t_2^* | \mu_2, \sigma, c_2) = \frac{1}{\sigma}\left[\ell n\left\{\frac{\tau_3 + c_3}{c_3}\right\} - \mu_3\right]. \qquad \text{(F-11)}$$

Hence, for t_3^* we have

$$t_3^* = \tau_3 + \Delta t_3$$

$$= c_3\left[e^{k(T_3^m - T_1^m)} - 1\right] + c_3 \sum_{i=1}^{3} \frac{1}{c_i} e^{k(T_3^m - T_i^m)} \Delta t_i \ . \qquad \text{(F-12)}$$

Continuing in this manner we obtain

$$t_n^* = c_n\left[e^{k(T_n^m - T_1^m)} - 1\right] + c_n \sum_{i=1}^{n} \frac{1}{c_i} e^{k(T_n^m - T_i^m)} \Delta t_i \ . \qquad \text{(F-13)}$$

Conclusion

For an arbitrarily specified sterilization time, t, $P_s(t)$ is expressible in the form

$$P_s(t) = 1 - \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\alpha(t^* | \mu, \sigma, c)} e^{-x^2/2} \, dx \qquad \text{(F-14)}$$

where

$$\alpha(t^* | \mu, \sigma, c) = \frac{1}{\sigma}\left[\ell n\left(\frac{t^* + c}{c}\right) - \mu\right] \qquad \text{(F-15)}$$

$$t^* = c\left[e^{k(T^m - T_1^m)} - 1 + \sum_{i=1}^{n} \frac{1}{c_i} e^{k(T^m - T_i^m)} \Delta t_i\right] \qquad \text{(F-16)}$$

and where μ, σ and c are the distribution parameters associated with temperature $T = T(t)$. The time intervals Δt_i are selected to insure that $t = t_n$. Note that for the special case where c does not vary with temperature

$$t^* = c\left[e^{k(T^m - T_1^m)} - 1\right] + \sum_{i=1}^{n} e^{k(T^m - T_i^m)} \Delta t_i \ . \qquad \text{(F-17)}$$

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APPENDIX G

Feasibility of a Stochastic Diffusion Model For Microbial Survival in Heat Sterilization

A rationale exists in favor of a physical diffusion model for describing the resistance of microbial spores subjected to heat sterilization. Although the destruction of a spore may involve chemical reactions, there is evidence that a non-chemical mechanism (i. e., a physical process) precedes the final destructive mechanism. Both I. J. Pflug and R. Angelotti (1, 2) direct attention to the observed dependence of microbial resistance on the moisture content, or water activity, of the spore prior to and during heating. In particular, Angelotti points to the increased dry heat resistance observed when spores are encapsulated within inert solids or trapped between mated surfaces prior to heating, indicating the possibility that this is related to the retention of moisture within the spore. Additional experimental evidence of interest in this connection was provided by Gerhardt and Black (3), who tested the permeability of spores to a glucose solution. They found that both germinated and heat-inactivated spores display an increased permeability to the glucose solution. Viable spores had a much smaller permeability. These results suggest that the outward diffusion of moisture through the spore walls, induced and accelerated by increased temperature, may be the key physical mechanism leading to the inactivation of spores.

An interesting connection between the diffusion process and the log-normal survival distribution can be developed on conjectural grounds. To see this, let x_o denote the "thickness" of a particular spore wall. Also, assume that the velocity of particles of moisture penetrating the wall (in either direction) is of the form:

$$v = v_o \, e^{-\alpha x} \qquad \text{(G-1)}$$

where x is the depth of penetration into the spore wall. This is a reasonable relation to consider if penetration is assumed to take place via a diffusion process

in the spore wall. From this we see that the time required for wall penetration is given by

$$T = \int_0^{x_o} \frac{dx}{V(x)} = \frac{e^{\alpha x_o} - 1}{\alpha v_o} \qquad \text{(G-2)}$$

Since the velocity on leaving the wall is substantially smaller than upon entering, $e^{\alpha x_o}$ must be much greater than 1. This permits the approximation

$$T = \alpha \frac{1}{v_o} e^{\alpha x_o} \qquad \text{(G-3)}$$

or

$$\ell n T = \alpha x_o + \text{Constant} \qquad \text{(G-4)}$$

It seems reasonable to assume that spore wall thicknesses vary within any given population. Moreover, if the wall thicknesses x_o are normally distributed over the total population then the time T required for desiccation of the spore is log-normally distributed. In form, this is consistent with empirical data on the microbial survival characteristics.

The above considerations provide justification for further exploration of an analytical model based upon the diffusion of moisture between spores and their surrounding environment. Such a development should take into account the various experimental configurations normally employed in related laboratory experiments, e.g., open, closed and intermediate systems. This will provide a basis for evaluating the resulting model in terms of available experimental data.

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